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ROBERT LARSSON
Editor of »Hereditas»
3 Adelgatan, LUND, SWEDEN.

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A CASE OF ASYNDESIS IN PICEA ABIES

BY *ENAR ANDERSSON*

INSTITUTE OF GENETICS, LUND, AND FOREST TREE BREEDING STATION, BRUNSBERG, SWEDEN

I. INTRODUCTION.

SOME investigations have treated in detail the embryology and fertilization of the Gymnosperms (cf. SCHNARF, 1933). Several have also dealt with the somatic chromosome numbers of these plants (cf. TISCHLER, 1926/1927, 1931, 1935/1936 and 1938, as well as A. and D. LÖVE, 1942), but few have been published respecting their chromosome behaviour at meiosis. The chromosome numbers of the Gymnosperms, in contrast to the condition in the Angiosperms, are remarkably constant even between families and differentiated genera. Polyploidy seems to occur very sparingly.

Most of the Gymnosperms have twelve chromosome pairs, or $2n = 24$. K. and H. J. SAX (1933) were able to determine the chromosome number in the endosperm, which in the Gymnosperms is haploid, for 53 species distributed over 16 genera among the Conifers. The now existing genera of coniferous trees number not fully 50. The investigators just cited found the basic number $n = 12$ in the majority of the Conifers. Species with deviating chromosome numbers are *Cryptomeria japonica*, *Taxodium distichum*, *Taivania cryptomerioides*, *Thuja occidentalis*, *T. orientalis*, *T. plicata*, *T. Standishii*, *Juniperus communis*, *J. virginiana*, *J. rigida*, and *Chamaecyparis Lawsoniana* with the basic number $n = 11$ or $2n = 22$, and *Pseudotsuga taxifolia* and the *Agathis* species (FLORY, 1936) with the basic number $n = 13$. For *Podocarpus* FLORY (1936) gives the following haploid numbers: *P. falcatus*, + 12, *P. macrophyllus*, + 19(—20), *P. neriifolius*, about + 19, *P. andinus*, about + 20, and for *P. chinensis*, + 20. According to TAHARA (1937), the haploid chromosome number of *Sciadopitus verticillata* is $n = 10$. *Pseudolarix* and *Juniperus chinensis Pfitzeriana* are tetraploid (K. and H. J. SAX, l. c.) with $2n = 44$. *Sequoia sempervirens* also seems to be polyploid, although no exact chromosome number has been determined. DARK (1932) found about 50 chromosomes in the root-tips of *S. sempervirens* and considers the species to be tetraploid, while K. and H. J. SAX (l. c.) approximately estimated the total chromosome number for the

same species at more than 40. BUCHHOLZ (1939 a and b) found the haploid number $n = 11$ in the sex-cells of *Sequoia gigantea* and the corresponding number $n = 22$ in *S. sempervirens*. The last-mentioned number, however, is given as only approximate. Finally, JENSEN and LEVAN (1941) determined the diploid chromosome number in root-tips of *S. gigantea* at $2n = 22$. After colchicine treatment of germinating seeds of *S. gigantea* they obtained tetraploid plants having the chromosome number $2n = 44$.

Among other Gymnosperms polyploidy has only been demonstrated in *Welwitschia* and *Ephedra*. *Welwitschia mirabilis* (FLORIN, 1932) has the chromosome number $2n = 42$ and among *Ephedra* plants *E. nebrodensis* var. *Villarsii*, *E. fragilis* var. *campylopoda* (GEITLER, 1929), *E. americana* and *E. equisetina* (FLORIN, 1932) have $2n = 14$, while *E. distachya* is tetraploid with $2n = 28$ (FLORIN, 1932). In *Picea* the chromosome number is stated by MIYAKE (1903) to be $2n = 24$, which has since been verified by K. and H. J. SAX (l. c.).

The present work chiefly deals with the course of meiosis in an asyndetic spruce as compared with that in normal spruces. Some investigations of pollen fertility and seed-setting after selfing and crossing in comparison with the seed-setting after free flowering as well as studies of the progeny raised from different spruces after free flowering will be published in Svensk Papperstidning, 1947. Asyndetic individuals do not seem to have been met with earlier within the Gymnosperms.

II. MATERIAL AND TECHNICAL METHODS.

1. MATERIAL.

At the Brunsberg branch station in Värmland of the Association for Forest Tree Breeding cones were collected during the autumn of 1942 and the spring of 1943, shortly after the free flowering period, from a large number of selected phenotypical élite trees of *Picea Abies* for estimation of the progeny. Sample trees of this type for selective breeding were taken at different levels of altitude and latitude. On the lands of the Billerud Company in the parish of Gunnarskog, West Värmland, cones from 1600 trees were collected in the winter of 1942—1943. During the work of seed extraction and cleaning of these cones it was found that one of the sample trees (spruce No. 181, Gröttvål)

had produced seeds belonging to a different order of size (Fig. 1). All the other trees sampled showed a good production of quite normal seeds. From 24 examined cones of spruce 181 there were obtained 7692 small seeds and 156 or 1,99 % of approximately normal size. Among 200 small seeds that were cut open 196 or 98 % were empty or had not developed any endosperm.

The small seeds were repeatedly placed for germination without a single seed being able to germinate. Among seeds of *a normal size-order*, 17 out of 100 or 17 % sprouted in the germinator at + 30° C.

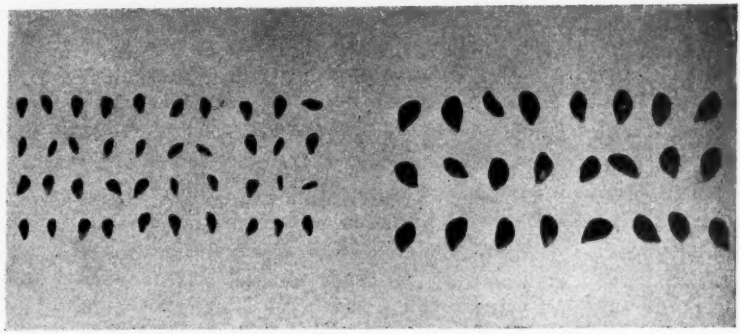


Fig. 1. Seeds of two different orders of size in the asyndetic spruce. — × ca. 1,5.

The 1000-grain weight for small and normally sized seeds from spruce 181 and for the average of the other selected trees within the stand is listed below:

1000-grain weight in grams, year 1943.

Abnormally small seeds from No. 181	0,88
Normal-sized » » » »	2,75
Mean size of seeds from other trees	3,46

Seed from No. 181 was sown in boxes in the spring of 1943, and the resultant seedlings were potted for root fixation. There was a great loss of plants in the boxes, about 30 % of the plants dying. Of 78 surviving offspring, about one-third were characterized by very feeble growth with short needles, and 16 died during cultivation in pots. In some of these plants it was possible to determine the somatic chromosome number.

The mother tree, No. 181, did not flower in the springs of 1943 and 1944. In the autumn of 1944, however, numerous male buds were put forth. In the middle of March, 1945, branches and male flowers were placed in greenhouses for forcing. They were put into glass jars containing water. Male inflorescences were irradiated with two 500 watt lamps every afternoon for four hours. After about 48 hours' irradiation it could be determined by fixation in acetic acid carmine that meiosis had commenced in the buds. For comparison branch and bud material was taken from six sample trees known to have normal seed setting.

In Värmland the meiotic division in spruce started in nature about April 13 in 1945. A large number of staminate inflorescences were then fixed of No. 181 as well as of 20 supposed normal spruce trees. These 20 trees also included the six previously mentioned spruces. The sample trees were derived from the following six localities: Nos. 108, 110, 112, 115, 125 and 140 from Brunsberg, Nos. 166, 220 and 221 from Gröttvål, Gunnarskog, Nos. 4, 17 and 121 from Fredros, Gunnarskog, Nos. 1, 16, 19, and 21 from Vägsjöfors, No. 1 from Lekvattnet, and Nos. 101, 102 and 103 from Höljes in northwestern Värmland. Buds from all the trees were fixed while meiosis was going on in nature. No disturbance whatever in the course of meiosis seems to have been occasioned through bud-forcing under irradiation in greenhouses at + 12 to + 16° C., buds from the six controls having shown the same normal course of division as buds taken direct from the control trees. On being forced in greenhouses the buds of spruce 181 exhibited the same disturbances at meiosis as when fixed direct in nature.

2. CYTOLOGICAL METHODS.

For root fixations use was made of MÜNTZING's modification of NAVASHIN's solution (MÜNTZING, 1932) as well as of LEVITSKY's solution (1931): 8 parts of 1 % chromic acid and 2 parts of 10 % formalin mixed immediately before use. Before staining, objects fixed in LEVITSKY's solution 8 : 2 were allowed to stand in a mordant of 1 % chromic acid for 12 to 24 hours. Both methods seem to be fully practicable and reliable for root fixations of spruce. At root fixing of offspring from No. 181 the jars with plants were placed on ice overnight for cooling. After being embedded in paraffin the root-tips were cut into 14—17 μ thick slices and stained with gentian violet.

For studies of meiotic divisions very beautiful stainings were obtained in acetic orcein by the method described by DARLINGTON and LA COUR (1942) as well as in aceto-carmine (K. and H. J. SAX, 1933, and ÖSTERGREN, 1942).

The best permanent preparations of meiotic divisions were obtained by means of the smear method. After smearing, the object glasses were changed over as quickly as possible to MÜNTZING's modification of NAVASHIN's fixative. Smear preparations

were then stained in gentian violet as well as in fuchsine-sulphurous acid (cf. DARLINGTON and LA COUR, 1942) and mounted in Canada balsam. After the smearing the object glasses ought to lie about 30—60 minutes or more in the above-mentioned fixative.

Pollen and stomatal size were determined in aceto-carmine + glycerine (1 : 1).

The drawings were made with the aid of Abbe's camera lucida and the micro-photographs were taken with a Zeiss spherolux camera.

To enable crosses to be made for breeding purposes between selected élite stocks in nature the Association for Forest Tree Breeding erected specially designed crossing-stages round a number of trees that had already been judged as regards progeny or were presumed to be good genotypes (cf. SYLVÉN, 1943). A crossing-stage of this kind was put up in April, 1945, round the asyndetic spruce 181 at Gröttvål, Gunnarskog (Fig. 2). With the aid of this scaffolding it was possible to take advantage to the flowering time of the tree to self a large number of female cones as well as to effect crosses with other spruces.

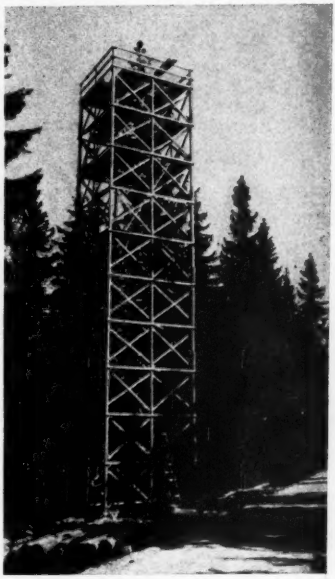


Fig. 2. Scaffolding round spruce No. 181 for crossing purposes.

III. NORMAL MEIOSIS.

As previously mentioned, for comparison with meiosis in the asyndetic spruce male inflorescences were fixed from 20 spruce trees that presumably possessed a normal meiotic division. Three of these spruces show disturbed meiotic division. This is described later in this work. The other 17 trees have a completely regular meiosis.

During leptotene and zygotene the chromosomes are much elongated and lie scattered at random inside the nuclear membrane. In leptotene the chromosome threads therefore form a reticular bunch, the individual threads of which are usually difficult to distinguish along their whole length.

It is clearly to be seen that the chromosome threads are of granular structure. At pachytene the threads are powerfully reduced in number and are paired. After parallel conjugation a relatively powerful contraction takes place of the chromosomes.

Fig. 3 *a*. Diplotene in normal spruce. — × ca. 900.

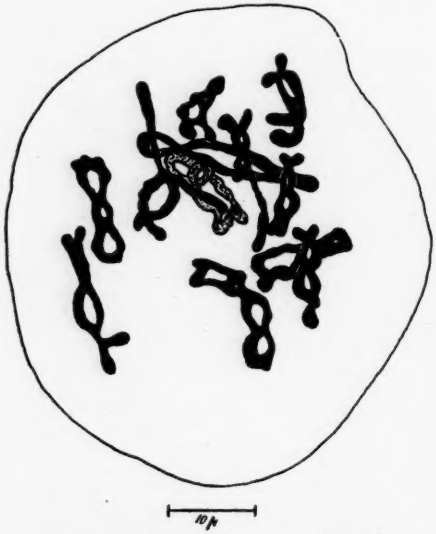


Fig. 3 *b*. Diplotene in normal spruce. — × ca. 1300.

During pachytene the chromosomes are distinctly paired, chromomere for chromomere. The chromosomes then increase in thickness, which first manifests itself by a swelling of the chromomeres. A contraction of the chromosomes occurs later in the middle of diplotene. The chromosomes separate more and more clearly from one another along their whole length, except at certain contact-points, as will be seen in Fig. 3 *a*. In Fig. 3 *b* the number of chiasmata are represented for each of the 12 bivalents. The mean number of chiasmata per bivalent is at this point of time $2{,}81 \pm 0{,}04$, as seen in Table 1. Most of the chiasmata are interstitial and persist as such until early metaphase I. Any terminalization of chiasmata is, as we shall see later, either very indistinct or, probably, non-existent.

In the Angiosperms the nucleolus is generally dissolved during the end of the diakinetic stage (DARLINGTON, 1937). The occurrence of the nucleolus during the greater portion of diakinesis, as well as its gradual disappearance during late diakinesis, may therefore be denoted as very characteristic of this stage in

TABLE 1.

Tree No.	No. of cells examined	Division stage	Distribution of bivalents with following number of chiasmata					Mean chiasmata per bivalent
			1	2	3	4	5	
112	30	Diplotene		149	150	43	18	$2{,}81 \pm 0{,}04$
112	30	Diakinesis		151	148	45	16	$2{,}79 \pm 0{,}04$
112	24	Metaphase I	3	118	115	43	9	$2{,}78 \pm 0{,}04$
101	30	»	9	158	129	53	11	$2{,}72 \pm 0{,}05$
220	30	»	6	153	136	54	11	$2{,}75 \pm 0{,}04$
121	30	»	2	155	141	52	10	$2{,}76 \pm 0{,}05$
4	24	»	2	118	116	45	7	$2{,}78 \pm 0{,}05$
16	20	»	5	102	99	28	6	$2{,}70 \pm 0{,}05$
19	20	»	6	96	103	27	8	$2{,}73 \pm 0{,}05$

the Angiosperms. In *Picea Abies*, on the other hand, the dissolution of the nucleolus seems to take place considerably earlier and as a rule is going on at the beginning of diplotene. At the end of diplotene and during the diakinetic stage cells with a nucleolus are only rarely found.

All chiasmata remain unchanged in diakinesis. Among other signs showing this is the chiasma frequency in 30 analysed cells of spruce 112. In these cells the mean number of chiasmata per bivalent was found to be $2{,}79 \pm 0{,}04$ during diakinesis. The same spruce showed a mean number of chiasmata per bivalent for the same number of cells during diplotene as was earlier observed, viz. $2{,}81 \pm 0{,}04$. The difference between these means is not statistically significant.

In Fig. 4 a cell of spruce 112 is seen during diakinesis. The chromosomes are much contracted and the bivalents are of about the same order of magnitude as during metaphase I. Here the number of chiasmata per bivalent is 2,83. This mean varies somewhat from cell to cell. The mean number of chiasmata per bivalent as between cells has varied during diakinesis between 2,67 and 3,0. The intra-cell variation is definitely greater and ranges from 2,0 to 5,0 chiasmata per bivalent. Solitary bivalents with one chiasma have been found only in metaphase I (cf. Table 1). Bivalents with more than 5 chiasmata have not been observed. As is evident from most of the illustrations, the bivalents vary in length. All the chromosomes have median or submedian centromeres (cf. also K. and H. J. Sax, 1933).

Metaphase I is illustrated by Figs. 5 and 6. Sometimes it occurs that a bivalent reaches the equatorial plane too late to range itself among the others. Such a late-comer will often be excluded from the common divisional plane of the other bivalents. This is probably due

to there being insufficient space on the metaphase plate. Under such circumstances this bivalent must divide alongside the equatorial plane of the cell.

The relative sizes of the bivalents during metaphase I and the number of chiasmata per bivalent and their positions as well as the positions of the centromeres are illustrated in Figs. 5 and 6. In both figures the bivalents are drawn separately — without taken account

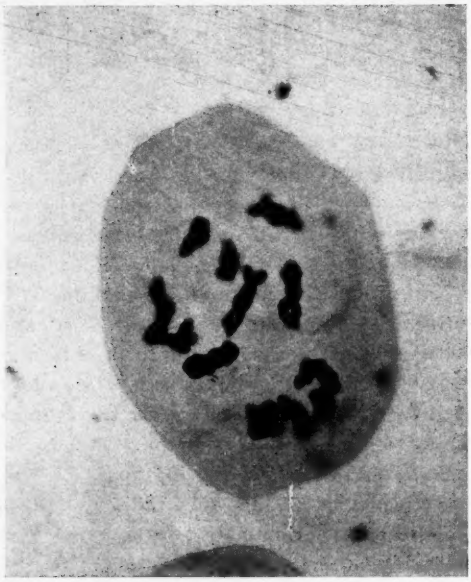


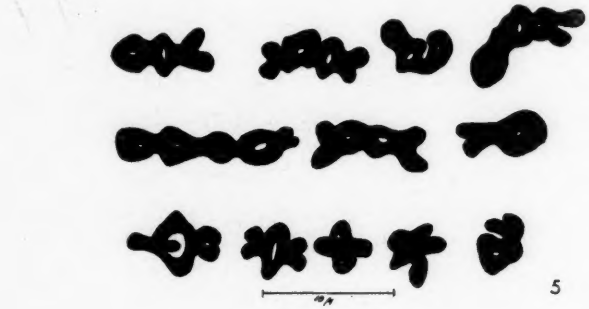
Fig. 4. Diakinesis in normal spruce. — × ca. 900.

of their relative positions in the cell. Each figure contains, however, a complete set of bivalents.

Figures showing the chiasma frequency during metaphase I are given in Table 1. The number of chiasmata per bivalent ranges from $2{,}70 \pm 0{,}05$ to $2{,}78 \pm 0{,}05$. As will be seen, terminalization of chiasmata does not occur in proportion to the number of chiasmata per bivalent during diplotene and diakinesis. From the table it is also evident that the variation between trees in respect of the mean number of chiasmata per bivalent is not statistically significant.

According to DARLINGTON (1937), a minimum terminalization may partially depend upon the size of the chromosomes, for in *Stenobothrus* amongst others he

observed that the small chromosomes invariably terminalize completely whereas the process is slow and incomplete in the case of the large chromosomes. On the other hand, in other species, e. g. of *Allium*, no such correlation seems to exist between terminalization and chromosome size. From a comparison of different species



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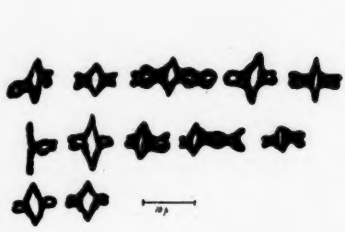

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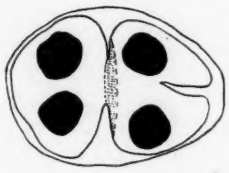

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Figs. 5—6. Bivalents drawn separately during metaphase I in normal spruce. — Fig. 7. Early metaphase II. Division wall being laid down centrifugally during metaphase II in the asyndetic spruce. — Fig. 8. Laying down of division walls in microsporocytes in normal spruce. — Fig. 9. A cell-plate has developed and tendency to »Furchung» or centripetal wall formation is already present during metaphase II in the asyndetic spruce. — Fig. 5, × ca. 2100. Figs. 6—7, × ca. 900. Figs. 8—9, × ca. 450.

DARLINGTON therefore infers that other factors, such as longitudinal contraction and repulsion of the centromeres, exercise a great influence on terminalization quite independently of chromosome size. MATHER (1940) has advanced very strong reasons for assuming that chiasmata always strive for definite positions and that no visible change in position occurs after chiasma formation has taken place, except in those cases in which complete terminalization occurs. He therefore distinguishes between chiasmata that terminalize and such as remain interstitial. He says: »Thus, in general, terminalization is an all or none process».

First anaphase and the succeeding stages of division in the spruce proceed as normally and schematically as the prophase and first metaphase divisions. Sometimes there persist for a time terminal connections or chromatin bridges between one or a pair of chromatids after the others have separated. Fragments or chromosome bridges with fragments have never been observed. When the chromosomes have reached the poles two daughter nuclei invested by nuclear membranes are formed in a normal manner. After that, the daughter nuclei enter into a resting stage, during which the chromosomes are difficult to distinguish and in course of time assume an appearance which to some extent resembles that of the mother-nucleus before division. In many daughter-nuclei during telophase I there appear 3—10 small globular bodies. Probably, extra nucleoli are concerned here similar to those described in *Pisum* by HÅKANSSON and LEVAN (1942). These bodies appear during telophase I in most of the pollen mother-cells, though in varying number. During early interkinesis most of them probably coalesce. By the end of interkinesis the number has often been reduced to two or three nucleoli, which later entirely disappear before second prophase.

No support has been found by me in the spruce for the hypothesis that the interkinetic stage is especially prolonged in time in the Gymnosperms as compared with other meiotic stages. TISCHLER (1943) states with respect to the length of the interkinetic stage: »In der älteren Karyologie wird angegeben, dass in gewissen Pflanzengruppen wie Pteridophyten und Gymnospermen die Neigung zu langen Interkinesen vorherrschen kann. Belege dafür boten *Botrychium*, *Helminthostachys*, *Equisetum*, *Larix*, *Taxodium*, *Pinus*, *Thuja* u. a.». TISCHLER himself, however, considers that external time factors probably have a greater influence than the genetic on the course of interkinesis.

Second division follows the course of an ordinary mitosis. The chromosomes arrange themselves in regular plates and divide lengthwise, which in reality means that only the centromeres divide, since

the chromosomes are already divided into two chromatids. Second anaphase likewise proceeds quite normally and gives rise to four tetrad nuclei. As a rule the tetrads lie in the same plane.

IV. MICROSPORE FORMATION IN PICEA ABIES.

In *Picea Abies* the tetrad formation follows the simultaneous scheme as in *Pinus silvestris* (VOGEL, 1936). In the asyndetic spruce the simultaneous tetrad division is also the most commonly occurring, although the succedaneous course of division has also been observed in a number of cells. The literature contains very divergent statements concerning tetrad division in the Gymnosperms. A collocation of the microsporogenesis in the Gymnosperms has been made by SCHNARF (1933, pp. 6—10). Data relating to the production of microspores within Pinaceae are mainly confined to *Pinus* and *Larix* and are lacking as regards *Picea.*

Pinus and *Larix* represent the simultaneous type (NEMEC, 1910; DÉWISÉ, 1922; PROSINA, 1928; STRASBURGER, 1895; FERGUSON, 1904; JURANYI, 1882). Other, rather deviating particulars have been given by COULTER and CHAMBERLAIN (1917) respecting *Pinus laricio*, the division of which may take place both simultaneously and succedaneously. According to COULTER and CHAMBERLAIN, the last-mentioned division is the commonest. In *Larix europaea* var. *pendula* the microspore formation is also reported to be able to occur either according to the simultaneous or the succedaneous scheme.

As a rule the tetrad division proceeds rapidly in *Picea Abies*, as does also wall formation between the tetrad nuclei.

Following the heterotypic division certain thickenings in the plasm are now and then seen in telophase I as well as traces of a cell-plate, though this plate disappears and is not laid down until anaphase II or after the tetrad nuclei have been formed. In spruces having normal meiosis the pollen mother-cells follow the simultaneous scheme. In the asyndetic spruce dividing walls are formed in some cases during metaphase II. Of 633 cells in metaphase II, 29 or 4,6 % had developed dividing walls. No compartment walls are formed immediately after the heterotypic division; however, they are laid down during metaphase II. Fig. 7 shows a cell during early metaphase II, where the dividing walls are under construction. The compartment walls growing out during metaphase II seem as a rule to arise by a new formation of cell-plates in the middle of the cell, these growing out centrifugally towards the periphery of the cell (Fig. 7).

Generally the cell-plate is thickest in the middle of the cell and therefore has the appearance of a very small and pointed nuclear spindle. However, the incomparably commonest way in which new dividing walls seem to be formed is by the joint action of cell-plates and cell-constrictions or so-called »Furchung» (Fig. 8). A similar wall formation in microspore-cells appears to have been found by MANN (1924) in *Ginkgo biloba*. Frequently the first cell is divided into two »dyad cells», which in their turn are divided by cell-plate formation and »Furchung». Sometimes no complete cell-plate is seen at this division, the dividing walls appearing to arise solely as a result of »Furchung». *It is however most probable that a conjoint action of cell-plates and cell-constriction is always involved in the construction of the dividing wall.* In Fig. 9 a cell of spruce 181 is represented during metaphase II, where a cell-plate has been formed and a tendency to cell-constriction or centripetal wall formation exists.

With regard to the laying down of the separating walls of the microspore cells in *Pinus silvestris*, VOGEL (1936) states among other things: »Während der zweiten Teilung wurde die ganze Zelle mit zusammenhängenden, unregelmässig verlaufenden Plasmafäden erfüllt. Hieraus entstanden zarte Wände, die die Mikrosporen entstehen liessen und voneinander abschlossen . . . Bald verdickten sich diese Wände und vereinigten sich mit der inneren Wand der Sporenmutterzelle. Es erfolgt weitere Verdickung der äusseren Wand, mit der eine Verstärkung der die Tochterzellen trennenden Wandung parallel geht». VOGEL's exposition gives the impression that, to begin with, the thin dividing walls hang freely in the cells, which is probably not the case.

V. COURSE OF MEIOSIS IN THE ASYNDETIC SPRUCE No. 181.

1. PROPHASE.

During early prophase in spruce 181 no major deviations are found from the course of division in a normal spruce. Leptotene and zygotene seem to proceed normally. Chromosome pairing in early pachytene is approximately as complete in the asyndetic spruce (Fig. 10) as in any spruce with normal reduction division. Even during late pachytene and early diplotene there is a very distinct difference between the asyndetic type and the normal. All previously formed spirals between paired chromosomes within the bivalents seem to regress and the

chromosome pairs gradually fall apart and numerous univalents begin to appear. It is therefore clear that on the whole there has been a failure in the formation of real chiasmata. At the point when the chromosome contraction becomes noticeable during diplotene earlier contact-points within the chromosome pairs seem to vanish. Signs of chiasmata or crossing-over points between paired chromosomes are observed in solitary cases during diplotene, but it is impossible to

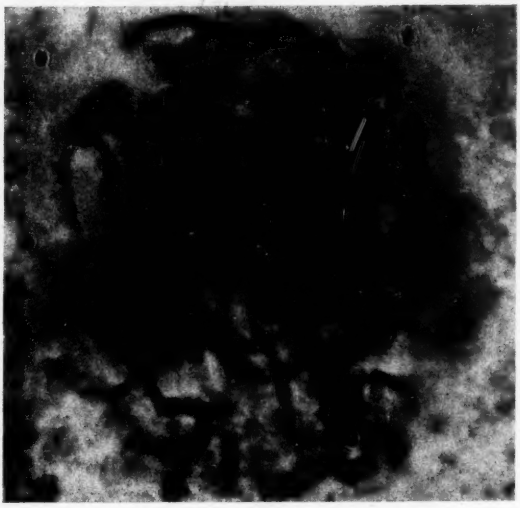


Fig. 10. Early pachytene in the asyndetic spruce. — × ca. 3000.

determine whether in such cases it is a question of real chiasmata or merely contact-points, or relational coiling.

At diakinesis the difference between the asyndetic and normal spruce types is very distinct. Often 24 univalents are observed in every pollen mother-cell of spruce 181 (Fig. 11) or cells with 1—3 bivalents and respectively 22 and 18 univalents. Multivalents or coalesced chromosomes also occur in low frequency. Almost all the bivalents that occur have one chiasma. More rarely ring bivalents or bivalents with two chiasmata are observed. These chiasmata are generally terminal. Diakinesis passes over into metaphase I without any great noticeable changes.

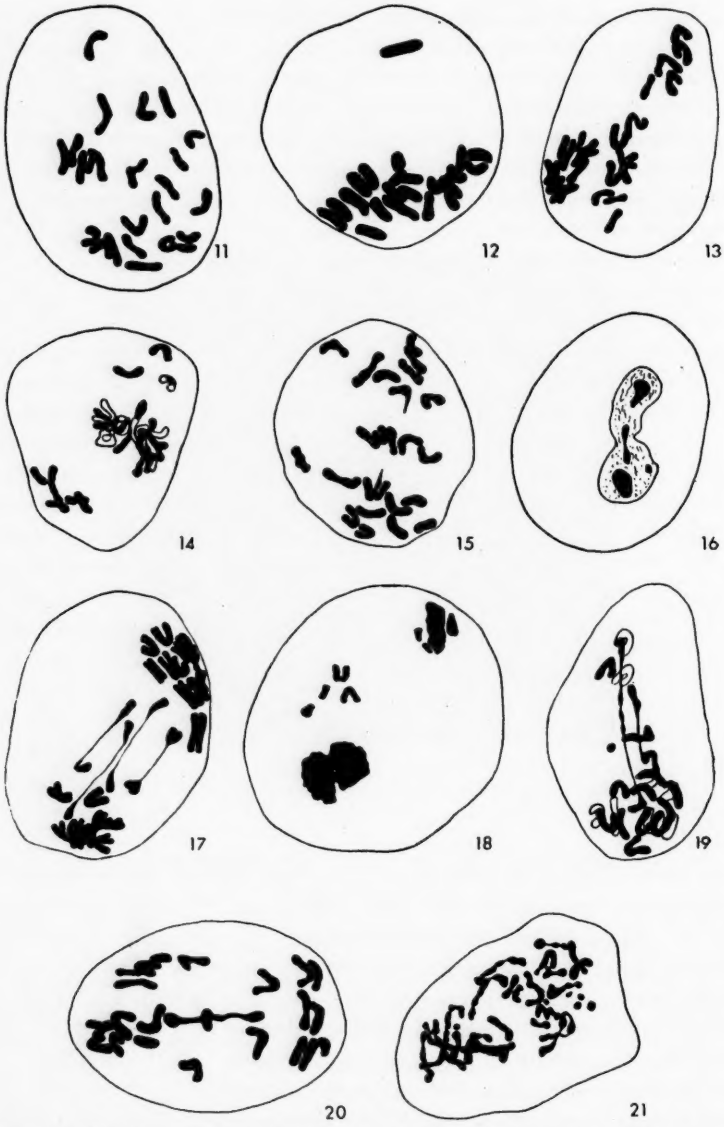


Fig. 11. Diakinesis — early metaphase I in the asyndetic spruce. — Figs. 12—14. Metaphase I. — Fig. 15. Anaphase I. Some univalents remain at the equator after the paired chromosomes of the bivalents have separated. — Fig. 16. Restitution

2. FIRST DIVISION.

During early metaphase I the chromosomes in cells with solely or almost solely univalents lie scattered around in the cells. In cells with multivalents, on the other hand, almost all the chromosomes are clustered together. During metaphase I all kinds of transitional forms appear, and the chromosomes are mostly arranged more or less asymmetrically to the equatorial plane of the cell and the longitudinal axis of the spindle.

In the asyndetic spruce the different meiotic stages of the first division are not so sharply differentiated as in normal meiosis. Earlier it was pointed out that the transition from diakinesis to metaphase I is less distinct in the asyndetic spruce. Almost as diffuse is the transition from metaphase I to anaphase I in spruce 181. This is largely due to the fact that in many cells only or almost only univalents occur. The univalents are scattered all over the cell. As a rule, they do not first of all arrange themselves into a nuclear plate in the equatorial plane of the cell but migrate direct to the poles (Fig. 12). Moreover, this separation toward the poles mostly takes place successively. The meiotic divisions in the normal and asyndetic types also differ in respect of time. The first division takes about double as long in the asynaptic spruce. There is however a smaller difference in time between the second divisions of the two types. Out in nature the meiotic division of the normal type goes on for 2—3 days. The corresponding time for spruce 181 is 4—6 days.

A strong influence is naturally exercised on the division intensity by the temperature and light conditions. When male inflorescences of spruce are being forced in a greenhouse, light seems to have a greater effect than heat on the rate of division. In strong sunshine the second division of spruce in nature under a temperature of about + 15° C. may go on for two or three hours. During metaphase I one or more bivalents occur in about two-thirds of the total number of pollen mother-cells. More than five bivalents per cell is very rare. With the exception of some rod bivalents with interstitial and median chiasmata all bivalents have terminal chiasmata. The majority of these consist of rod bivalents with one chiasma. In solitary cells multivalents have been

nucleus. — Fig. 17. Anaphase I with misdivision of univalents. — Fig. 18. Late anaphase I—telophase I with fragments, which have probably arisen through misdivision. — Figs. 19—20. Late metaphase I. — Fig. 19 shows a »bridge» with a fragment. — Fig. 20. Bivalent with interstitial chiasma. — Fig. 21. Late diplotene. Cell with much fragmentation. — × ca. 800. (The cell in Fig. 12 was fixed in acetic orcein.)

observed. Among 500 examined pollen mother-cells multivalents occurred in seven or in 1,4 % of the total number of pollen mother-cells. In a number of cells the chromosomes are fased together and cannot therefore be counted. The number of bivalents per cell has been counted in 1164 cells. A division of the cells with respect to the number of rod and ring bivalents per cell and the mean number of chiasmata per bivalent within the different groups is given below.

Number of bivalents per cell	0	1	2	3	4	5	6	7	8	Total number of cells
» » cells with 0—8 rod bivalents ..	456	226	220	92	40	33	13	3	5	1088
» » cells with rod and ring bivalents			18	16	8	11	3	1	3	60
» » ring bivalents in these cells			(18)	(19)	(13)	(22)	(7)	(3)	(13)	—
» » cells with solely ring bivalents ..		14	2							16
Mean number of chiasmata per bivalent		1,06	1,05	1,06	1,07	1,10	1,07	1,11	1,20	
Number of cells	456	240	240	108	48	44	16	4	8	1164

From the table it will be seen that the number of chiasmata per bivalent increases on account of the fact that the percentage of ring bivalents per cell rises with increased number of bivalents per cell. The mean number of chiasmata per bivalent in the cells examined is 1,07. Real chiasmata thus appear to be formed in the asyndetic spruce, although the bivalent frequency is very low. In the examined pollen mother-cells bivalent formation was entirely absent in 39 %. In other cells examined there were 1644 bivalents out of 8496 possible ones. Expressed in percentage, the bivalent formation in cells with one or more bivalents will be 19,35 % of the number of possible bivalents. Of this frequency 113 out of 1644 bivalents, or 6,87 %, consist of ring bivalents. In all examined pollen mother-cells from No. 181 the frequency of bivalents is only 11,77 % of the number of possible bivalents, or comparable with the bivalent frequency in spruce trees having normal meiosis. A division of this frequency into rod and ring bivalents gives 10,96 % rod bivalents and 0,81 % ring bivalents. Almost all ring bivalents have terminal chiasmata, the number possessing interstitial ones being insignificant. A single ring bivalent was observed with three chiasmata; all the rest had two.

Besides ring bivalents, metaphase I and anaphase I also exhibit ring-shaped univalents that have terminally attached chromosome-ends

and thus form a closed ring. These ring univalents were observed in 37,2 % of the number of pollen mother-cells counted. The idea readily suggests itself that these ring univalents may be iso-chromosomes which have arisen through misdivision in one of the parental trees of the asyndetic spruce. Such iso-chromosomes have double segments and are therefore able to pair. A summary of works on iso-chromosomes is given by MÜNTZING (1944, pp. 245—246; cf. also 1945). In No. 181 some cells contain one to three ring univalents, which makes it highly improbable that iso-chromosomes are concerned in this case. Therefore, a more likely explanation is that the ring univalents present have merely stuck together and that the attachment between the chromosome-ends is of a heterochromic nature. The frequency of ring univalents per cell is as follows:

No. of ring univalents per cell	0	1	2	3	4	Total number of cells
No. of pollen mother-cells	302	141	33	5	0	481

The second division is also marked by the occurrence of solitary ring univalents, which makes it still more improbable that this is a case of iso-chromosomes.

During metaphase I the relative positions of the chromosomes in the cells exhibit highly variable metaphase pictures. The chromosomes may either be evenly distributed (Fig. 11), heaped in the middle of the cell or at the cell-wall (Fig. 12), arranged in metaphase plates, which may be more or less displaced from the equatorial plane of the cell, or strewn at random round the nuclear spindle (Fig. 13). The chromosome configuration in Fig. 13 has either been caused by a tripolar or a crescent-shaped spindle. This latter alternative would seem to be the more probable in this case. Tripolar spindles occur in low frequency in normal spruces, while abnormally stretched and semilunarly curved spindles are only met with in the asyndetic type (cf. also DARLINGTON, 1937, p. 415). These different metaphase pictures are a direct consequence of the *behaviour of the univalents during metaphase I*. When no bivalents occur in the cells, the univalents mostly lie strewn at random round the cell or along the nuclear spindle. The univalents do not as a rule arrange themselves in a metaphase plate but preferably migrate direct to the poles. Their distribution on the two poles certainly seems to take place entirely at random (Fig. 12), although there is poor agreement with the theoretically expected distribution. When the chromosome groups are well separated, two interkinetic nuclei arise.

Occasionally some univalents may remain in the equatorial plane of the cell. These afterwards migrate either to one of the poles to enter into the two daughter-nuclei or to remain there and form an extra interkinesis nucleus. It also occurs that the univalents retarded at the equator join the two other chromosome groups and give rise to one or more restitution nuclei. Restitution nuclei occur however in very low frequency in spruce 181.

In the presence of bivalents the majority of the univalents also seem to arrange themselves in a metaphase plate. Together with the bivalents they form a common plate at the equator (Fig. 14) or at the side of the latter. Univalents associated with real bivalents seem to have a tendency to behave as »bivalents» and to form a common plate with them. Those univalents which are not included in the metaphase plate generally remain in their original positions during division of the bivalents. Most frequently, however, one or more univalents are observed at or near the poles at the time the bivalents divide. These univalents must either have reached the poles before formation of the metaphase plate or have passed to the poles after the rest of the chromosomes had arranged themselves in a plate. When the paired members of the bivalents have separated and commenced their migration to the poles, the univalents also begin to shift towards one of the poles. During this anaphase migration other univalents scattered around in the cell also make their way to one of the two poles.

Only in a few pollen mother-cells do one or more univalents remain in the equatorial plane of the cell and divide after the paired chromosomes of the bivalents have separated (Fig. 15). After that, the two univalent halves or the chromatids either pass each to its pole and are included in the daughter-nuclei or remain at the equator and are marked off from the anaphase nuclei in small micronuclei. These split univalents may serve as connecting links between the anaphase nuclei and give rise to restitution nuclei (Fig. 16) if the separation of chromatids or possibly of only longitudinally divided univalents with undivided centromere takes place before the daughter-nuclei have invested themselves in a cell-membrane.

Univalent splitting seems to be rare in the pollen mother-cells of absolutely asynaptic Angiosperms with one or more bivalents. Only among hybrids between *Solanum tuberosum* × *S. curtilobum* (LAMM, 1941) and certain inbred plants with absolute asynapsis in *Alopecurus myosuroides* (JOHNSSON, 1944) have split univalents been found. On the other hand, such are common at anaphase I in plants with partial or rather weak asynapsis. This is the case in e. g. *Hordeum* (EKSTRAND, 1932),

Crepis (RICHARDSON, 1935), *Pisum* (KOLLER, 1938), *Populus* (JOHNSSON, 1940), and *Secale* (PRAKKEN, 1943). In *Picea Abies* split univalents occur in anaphase I not only in cells with one or more bivalents, which may be regarded as cells with partial asynapsis, but also and most frequently in cells that have no bivalents. The centromeres divide in anaphase I after the univalents have first oriented themselves in a regular metaphase plate.

During late anaphase I the univalents distributed to the poles also divide lengthwise with the exception of the centromeres. The chromatids seem to be distributed both regularly and irregularly to the poles — undivided univalents occasionally pass at the same time as divided univalents to both the poles. A similar case has already been described by LAMM (1941) in sterile hybrids from the cross *Solanum tuberosum* × *S. curtilobum*. Bivalents also seem sometimes to pass undivided to the poles. This is particularly the case when only one bivalent occurs per cell. Such cases of non-disjunction, however, occur very rarely. Figs. 17—18 illustrate indications to misdivision, which probably occurs in solitary cells. Fig. 17 shows some lagging univalents while division is in progress. Their centromere regions are filamentously drawn out into bridges, and the centromeres do not appear to be able to divide. The fragments appearing in Fig. 18 are probably products of misdivision. These four fragments have presumably arisen through the transverse division of the univalents. As in haploid rye (LEVAN, 1942), telocentric fragment-chromosomes have been observed in a number of cells during the second division.

Misdivision has been specially discussed and described by DARLINGTON (1939, 1940) in *Fritillaria kamtschatkensis*. Further cases have since been found in several materials, e. g., in *Godetia Whitneyi* (HÅKANSSON, 1940 a and b), *Secale* (LEVAN, 1942; PRAKKEN, 1943; MÜNTZING, 1944), and *Beta* (LEVAN, 1945). Even before 1939 cytological observations that can be interpreted as misdivision were made by several workers (cf. MÜNTZING, 1944).

As already mentioned, in a number of pollen mother-cells one or more split univalents make their appearance. The number of cells with split univalents and the number of split univalents per cell in 1164 cells are summarized below:

No. of split univalents per cell	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
No. of cells containing split univalents	10	6	3	5	2	1	0	1	1	0	3	2	0	0	4	2	0	0	2	1	0	2	1	2

Among 1164 pollen mother-cells split univalents were observed in 48 cells or in 4,1 % of the number of cells. In two cells all the uni-

valents were divided. The longitudinal division and the division of the centromeres in the univalents take place during anaphase I. Sometimes the division is incomplete in so far as the centromeres of a number of univalents remain undivided and only the chromatids separate. In the above collocation of split univalents, therefore, only such as possess divided centromeres have been included.

Besides the previously described anomalies in the meiotic division of the asyndetic spruce there occur considerably drawn-out bivalents with fragments — resembling inversion bridges with fragments — together with stickiness in a number of pollen mother-cells. Among the 1164 cells examined band-like drawn-out terminal or interstitial connections between some chromosomes have been observed in 161 cases or in 13,83 % of the number of examined and analysable pollen mother-cells. In these cells only one bridge per cell was found as a rule; in but few cases were 2—5 bridges per cell observed. Figs. 19—20 illustrate some bivalents and bridges of this kind. All these bivalents have one chiasma, as will be seen from the illustrations.

Judging from their positions and from the shape of the bivalents, the repulsion between the centromeres of the bivalents would seem to be of fully normal strength. Those parts of the bivalents which are located between the centromeres and chiasmata are mostly drawn out into long threads or chromosome bridges. In 13 of the 161 cells, or in 8,1 % of the number of cells with such bivalents, there are fragments of varying size together with bridges. This suggests the occurrence of inversion bridges, which would have arisen through crossing-over within the inversion coils. Thus, fragments occurring together with chromosome bridges would afford a strong support for the assumption that real inversion bridges are concerned in the thirteen cases just cited.

Arguing against this assumption is the absence of inversion bridges in normal spruces as well as the type of the bridges. There is reason to expect that the number of inversions in the asyndetic spruce is less than in the normal type owing to reduced chiasma formation. The general type of inversion bridge has not been met with in the asyndetic spruce. The fragments found together with the bridges need not necessarily be interpreted in this case as a sign of inversions.

Fragments also occur in a few cells without bridges. These fragments already arise at early diplotene through collapse of the chromosomes, which takes place in solitary cells. Fig. 21 illustrates such a transversal collapse of the chromosomes during late diplotene. In some cells showing much fragmentation there arise at metaphase I chromo-

some configurations which seem to be identical with the stickiness in *Zea mays* (BEADLE, 1932) and *Alopecurus myosuroides* (JOHNSSON, 1944).

During anaphase I the univalents in the asyndetic or asynaptic spruce appear to be distributed more or less at random to the two poles. In cells with solely or almost solely univalents there would therefore be reason to expect a completely random distribution of the chromosomes. The 12 : 12 distribution should quite naturally be the most frequently occurring one, while distributions such as 1 : 23, 2 : 22, 3 : 21 or 23 : 1, 22 : 2, 21 : 3, etc. ought to occur very sparingly if the distribution is to be binominal. To determine the chromosome distribution in different pollen mother-cells during anaphase I 186 cells were examined. The results are presented in Table 2. In making the chromosome counts the cell-pole that had been directed towards the north or the west part of the visual field in the microscope was invariably counted and recorded first. By this means the 17 : 7 distribution could be distinguished from the inverse one, 7 : 17, which theoretically ought to occur equally often as the former. This procedure is not necessary unless it is desired to record the distribution of the pollen mother-cells with regard to the chromosome distribution in the same manner as a binominal series.

TABLE 2. *Distribution of pollen mother-cells with varying or equal numbers of chromosomes at the poles during anaphase I in spruce 181.*

Distribution of chromosomes:	0:24	1:23	2:22	3:21	4:20	5:19	6:18	7:17	8:16	9:15	10:14	11:13
No. of pollen mother-cells:	1	3	5	3	5	4	7	9	10	12	9	15
Expected distribution:	0,00	0,00	0,00	0,02	0,12	0,47	1,49	3,84	8,15	14,50	21,74	27,67

Distribution of chromosomes:	12:12	13:11	14:10	15:9	16:8	17:7	18:6	19:5	20:4	23:1	22:2
No. of pollen mother-cells:	21	13	9	11	9	10	8	5	3	4	3
Expected distribution:	29,98	27,67	21,74	14,50	8,15	3,85	1,49	0,47	0,12	0,02	0,00

Distribution of chromosomes:	23:1	24:0
No. of pollen mother-cells:	4	3
Expected distribution:	0,00	0,00

Table 2 compares the found distribution of the pollen mother-cells with the binominal distribution $(p + q)^{24}$. As the probability that a certain chromosome will pass to a certain pole during anaphase I is $^1/_2$, the two probability values in this case have the value $^1/_2$. If the binominal $(^1/_2 + ^1/_2)^{24}$ is expanded and multiplied by the number of pollen mother-cells, the expected distribution will be obtained. There is undoubtedly a very bad agreement between the found and the expected distribution, and it is beyond all doubt that the two series are not identical. Not even a very powerful increase in the number of pollen mother-cells examined can in this case alter the agreement between the two series. This is most clearly evident from the direct probability values for the different classes obtained by expanding the binominal $(^1/_2 + ^1/_2)^{24}$. In this expansion we obtain in the numerator the following binominal series: 1; 24; 276; 2.024; 10.626; 42.504; 134.596; 346.104; 735.471; 1.307.504; 1.961.256; 2.496.144; 2.704.156; 2.496.144; 1.961.256; 1.307.504; 735.471; 346.104; 134.596; 42.504; 10.626; 2.024; 276; 24; 1. The common denominator for all terms will in this special case, when p and q are equal, then be 16.777.216. From this binominal distribution it is seen that the number of expected pollen mother-cells having, for instance, the 1 : 23 and 23 : 1 chromosome distribution will in each case be 24 out of 16.777.216 possible cases. According to the same distribution, the probability of finding cells with the 0 : 24 or 24 : 0 chromosome distribution is altogether only 2 cases out of 16.777.216.

We see from this that even with a very large number of examined cells the values for the extreme classes in the expected distribution will be 0. Utilizing the χ^2 method as a criterion for judging whether hypothesis and observation are in agreement with each other, the quotient between the difference of the series for the lowest classes and the expected distribution (0) will consequently be infinitely large. Apart from these distribution classes the observed and expected frequences are statistically quite different from each other. Only the χ^2 values for a couple of classes are necessary to give a P value of 0,001, which is immediately obvious from the two distributions, or perhaps still better from the following arrangement in which classes with in reality the same distribution have been grouped together:

0:24,	1:23,	2:22,	3:21,	4:20,	5:19,	6:18,	7:17,	8:16,	9:15,	10:14,	11:13,	12:12
11	8	7	8	9	15	19	19	23	18	28	21	
0	0	0,04	0,24	0,91	2,98	7,68	16,30	29,00	43,48	55,34	29,98	

Thus, the distribution of pollen mother-cells with varying or equal

numbers of chromosomes at the poles does not agree with the expected binominal distribution. The chief cause of this seems to be that the chromosomes have a certain tendency to appear in groups. These groups cause a considerable displacement in the expected binominal distribution of the chromosomes, behaving as they often do as one unit and entirely or partially migrating to one of the poles. This is especially the case in non-disjunction, which phenomenon *per se* implies an irregular distribution of the chromosomes. To this comes the fact that univalents plus one or a couple of bivalents will sometimes form a common group that often also behaves as one unit and passes to one or the other of the two poles.

3. INTERKINESIS.

During interkinesis more than two interkinetic nuclei frequently arise as a consequence of an irregular first anaphase separation. In several cases three or four nuclei per cell were observed and in one case five interkinetic nuclei among 274 examined cells. Cells with more than five nuclei occur rather sparingly. Still, in one cell 16 interkinesis nuclei could be distinctly observed, which shows that a single chromosome is able to form an extra nucleus. In cells having two nuclei the interkinesis nuclei are often of different size. As was expected, unicellular interkinesis also occurs in a number of cells [1]. Sometimes univalents and fragments in the plasm also appear outside the interkinetic nuclei.

4. SECOND DIVISION.

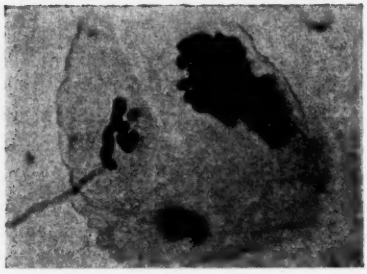
The second meiotic division is fairly normal in most of the cells, i. e. cells which contain solely double chromatids and are devoid of fragments. All other cells with one or more single chromatids, fragments, stickiness, three metaphase groups, or chromatin bridges are marked by a series of irregularities. This last group of cells consists of about 25 % of the number of pollen mother-cells. As a consequence of the irregular distribution of the chromosomes during anaphase I pollen mother-cells containing a highly varying number of chromosomes occur during the second division (Figs. 7, 9 and 22). A relatively good agreement exists between the distribution of univalents observed during metaphase I and early anaphase I and the number of chromosomes in the configurations during metaphase II. This correlation is brought out clearly by the following comparison.

[1] Out of 274 examined cells, restitution nuclei were formed in 24 cases or 8,76 %.

	Chromosome distribution among daughter-cells								
No. of pollen mother-cells:	0:24,	1:23,	2:22,	3:21,	4:20,	5:19,	6:18,	7:17,	8:16
In anaphase I	4	7	8	7	8	9	15	19	19
In metaphase II	15	5	6	8	7	6	12	15	14

No. of pollen mother-cells:	9:15,	10:14,	11:13,	12:12	No. of cells laid down
In anaphase I	23	18	27	21	186
In metaphase II	18	28	36	23	193

The number of cells having an unreduced chromosome number has increased substantially in comparison with what was the case after distribution of the univalents during metaphase I. This is due to the



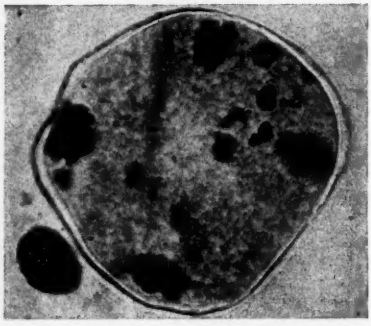


Fig. 22. Metaphase II in the asyndetic spruce. — Fig. 23. Telophase II with single chromatids remaining on the equator. — × ca. 800.

appearance of several restitution nuclei in anaphase I on account of the fact that lagging univalents in the equatorial plane and split univalents in certain cells have succeeded in establishing connecting links between the daughter-nuclei, which as a result have been united to one restitution nucleus. Some cells contain single as well as double chromatids. The single chromatids derive from the univalents divided in anaphase I. In metaphase II these chromatids are unable to divide and therefore remain on the equator during anaphase II (Fig. 23), to be eliminated from the tetrad nuclei and form micronuclei.

In most of the cells the chromosomes pass to the equator and form regular metaphase plates. In other cells they lie more or less scattered round the whole cell during metaphase II. The last-mentioned cells often seem to develop restitution nuclei and micronuclei. As in the first division, fragments appear in a few cells. These fragments may either

Fig. 24. A cell with unreduced chromosome number during anaphase II. — × ca. 1950.

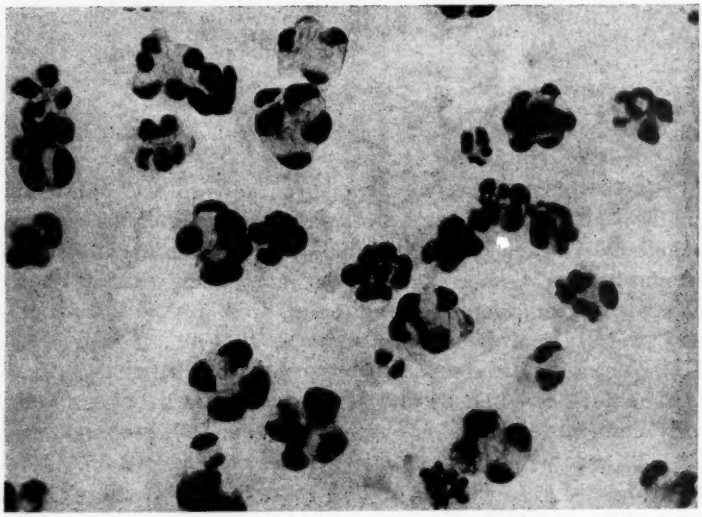


Fig. 25. »Pollen tetrads» from the asyndetic spruce. The variation in size among the pollen tetrads is remarkable and seems to admit of being put in association with the abnormality caused by stickiness. — × 120.

split and be included in the tetrad nuclei or remain unsplit in the equatorial plane.

A characteristic feature of cells with stickiness is, as in the first division, the presence of a number of chromatin clumps and fragments with chromatin bridges. However, a rather large number of free chromatin bridges seem to occur during the second division. Still, there are not so many unchanged or normal chromatids. The amount of chromatin may vary but is largely the same in the two daughter-cells. In solitary cells the chromatin masses in one or both daughter-cells seem to divide rather normally. Most frequently, however, cells characterized

Figs. 26—28. »Tetrads» with variable number of cells and nuclei in the asyndetic spruce. — × ca. 650.

by stickiness give rise to one or more micronuclei, which probably degenerate.

Cells in which the heterotypic division has failed to appear divide normally if all the chromatids are double. If some of the chromatids are single, these remain on the equatorial plate during anaphase and form micronuclei. A rare case of two chromatids with an undivided centromere during anaphase II is reproduced in Fig. 24. It is also possible that this univalent is on the way to the equator to divide there.

The »tetrads» in the asyndetic spruce are very irregular (Figs. 25—28). The number of nuclei and cells varies remarkably per »tetrad», as is evident from the illustrations as well as the following figures:

No. of nuclei and cells per »tetrad»:	2	3	4	5	6	7	Total
No. of »tetrads»:	8	24	298	36	6	3	375

A large number of the »tetrads» contain, as expected, extra micronuclei, the three first groups containing the most.

In the largest »tetrad» group the distribution of micronuclei is as follows:

No. of micronuclei per »tetrad»	0	1	2	3	4	5	6	7	8	Total
No. of »tetrads»	156	65	51	21	7	3	3	1	1	298

The number of microsporocytes with an unreduced chromosome number has decreased during the second anaphase, which is to be seen from the number of dyads as compared with the number of restitution

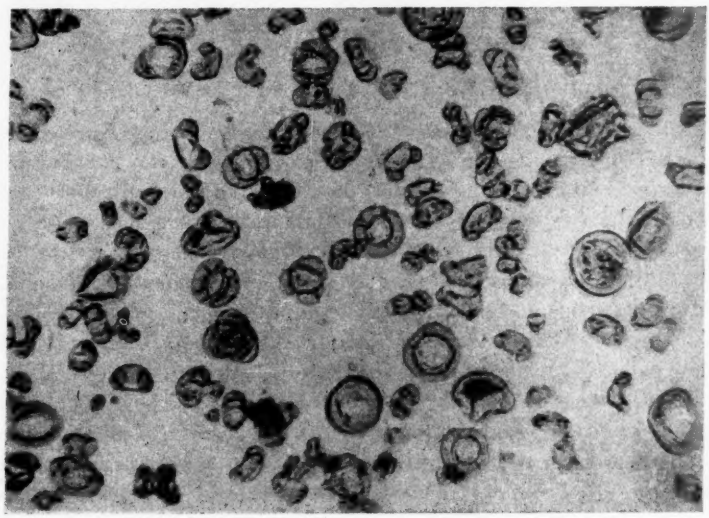


Fig. 29. Pollen from the asyndetic spruce. — × 120.

nuclei in the interkinesis stage. This decrease was due to the fact that these pollen mother-cells, unreduced during interkinesis, had contained single chromatids. During the second division such cells showed lagging chromosomes, which in most cases resulted in the arising of »triads» with nuclei of different size or »dyads» with micronuclei. Monads with only one nucleus were not observed. The remaining »triads» probably arose because one of the daughter-cells divided during the second division while the other daughter-cell formed a restitution nucleus. Dyads without micronuclei also occasionally exhibit variable nucleus-size. Two of the eight dyads contained respectively one and three extra micronuclei. The numbers of extra micronuclei in the 24 triads were:

No. of extra nuclei per triad:	0	1	2	3	4	5	Total
No. of triads:	8	6	3	4	2	1	24

In the 36 pentads the number of extra micronuclei was as follows:

No. of extra nuclei per pentad:	0	1	2	3	Total
No. of pentads:	26	7	2	1	36

Only one of the hexads contains a micronucleus. Stickiness and the irregular chromosome distribution in the asyndetic spruce give rise to a very irregular pollen, which varies both in size and form (Fig. 29).

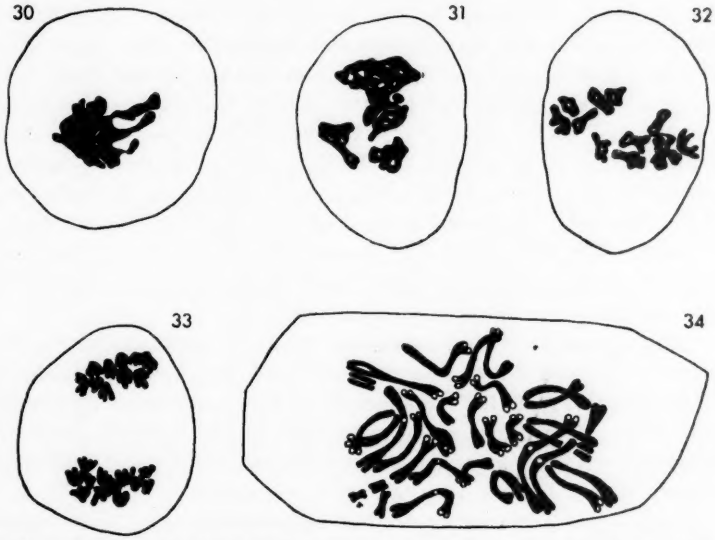
VI. THREE CASES OF STICKINESS.

The expression »sticky chromosomes» was first employed by BEADLE (1932) to denote a special abnormality of the chromosomes. This malformation consists in the chromosomes forming fused chromatin masses during meiosis and thus losing their individuality (cf. also BEADLE, 1937; JOHNSSON, 1944; DARLINGTON and LA COUR, 1940, 1945). Sticky chromosomes, moreover, seem to be considerably smaller than normal chromosomes. BEADLE found in maize a single recessive gene which caused stickiness in somatic divisions as well as to a still higher extent in meiotic divisions. As already mentioned, similar abnormalities occur in solitary microsporocytes in the asyndetic spruce. Stickiness was also found in another three spruces. In pollen mother-cells derived from these trees the chromosomes are fused into more or less irregular chromatin masses. The prophase stages are specially difficult to distinguish, the chromosomes in all these stages being drawn out into chromatin threads or coalesced into chromatin clumps. All kinds of transitional forms between normal or almost normal meiosis and complete stickiness appear.

In Figs. 30 and 31 two cells with pronounced stickiness are represented. Fig. 32 illustrates a cell having a normal or nearly normal meiosis. Each of these pollen mother-cells contains 12 bivalents, although these *are very powerfully contracted*. Of more occasional occurrence are cells with considerable fragmentation. This anomaly is in the asyndetic spruce more characteristic of cells exhibiting stickiness. On the other hand, asymmetric »bivalents» and »multivalents» with abnormal chiasmata occur. An apparently normal anaphase I with *greatly contracted chromosomes* is illustrated in Fig. 33. After anaphase I there usually follows a normal binuclear interkinesis. More

sporadically cells are found with three nuclei, nuclei of very variable size, or restitution nuclei.

The second division, like the first, shows different transitional forms. About half of the tetrad nuclei are of variable size, and micronuclei occur in most of the cells. After the second division several microsporocytes exhibit signs of degeneration.



Figs. 30—31. Cells with sticky chromosomes. — Fig. 32. Almost normal metaphase I in »sticky spruces». — Fig. 33. Anaphase I with greatly contracted chromosomes in a »sticky spruce». — Fig. 34. Somatic chromosomes from root-tips of offspring from the asyndetic spruce. Metaphase in a plant with $2n = 24 + 2$ f (four chromosomes are furnished with trabants). — Figs. 30—31, × ca. 1000. Fig. 34, × ca. 1650.

Two of these three spruces are derived from Brunsberg and one from Höljes. The growth locality of the spruces from Brunsberg is situated 70 metres above sea-level, while that of the spruce from Höljes is about 500 metres above sea-level. For fixation purposes the buds were collected direct from the trees. At the time of meiotic division in the spruces, which in 1945 occurred in Värmland between April 12 and 18, great differences in day and night temperatures are not uncommon. Especially on southern slopes the temperature may rise to between + 15 and + 20° C. on sunny days and fall during the night to between

— 10 and — 15° C. At Brunsberg a minimum temperature of — 8 to — 8,4° C. was registered on a couple of nights during the period in question. On the other hand, the temperature during the day kept between + 9,8 and + 14,1° C.

The possibility cannot therefore be ruled out that these cases of stickiness were caused by cold shock. In support of this hypothesis an experiment may be adduced that was carried out at Brunsberg on April 19, 1945. Branches bearing male inflorescences from No. 112 Brunsberg, which had been forced for twenty-four hours in a greenhouse at a temperature of + 18° C., were placed for the night out in the open. The minimum temperature during the night was registered at — 8,4° C. At the time the branches were moved out the pollen mother-cells were in process of division, and a control examination showed the majority to be in metaphase I. When the branches were taken in the following morning, most of the cells, after fixation, showed stickiness. The disturbances of meiosis following the cold treatment were at least as powerful as in the three »sticky spruces». Controls left in the greenhouses all showed normal meiosis.

Militating against the assumption that the stickiness in the three spruces mentioned is a consequence of temperature changes is the fact that two spruces from the same locality at Höljes and three from Brunsberg, which were fixed at the same time as the spruces found with stickiness from these localities, did not present any meiotic disturbances whatever.

From experience we know that the seed-setting of forest trees is especially poor in high positions, which means that self-juvenescence is jeopardized to a high degree within such forest areas. Now, does this poor seed-setting depend to some extent on modificative disturbances in the course of meiosis or to unfavourable climatical conditions during the pre- and post-fertilization periods? Unfortunately, we are not able at present to answer this and similar questions.

VII. PROGENY OF No. 181 AFTER FREE FLOWERING.

The 62 surviving plants after wind pollination of No. 181 exhibit highly varying height growth, growth habit, needle-length and stoma-size (cf. Tables 3 and 4). The variation within this progeny-group is strikingly large compared with that within the other progeny-groups of the same age from normal spruces. In habit some of the plants are reminiscent of witch-knot. This habitual variation is in all probability

TABLE 3. *Comparison between stomatal size, plant height and some chromosome numbers in progeny of spruce 181 after free flowering* (1 scale division = 2,47 μ).

		Stomatal length	Stomatal breadth	Plant height in mm.	2n
Plant No.	1	20,97 ± 0,11	13,68 ± 0,08	70	
	2	20,55 ± 0,13	12,63 ± 0,09	102	
	3	20,91 ± 0,13	12,97 ± 0,07	81	
	4	21,28 ± 0,10	12,69 ± 0,09	110	
	5	21,68 ± 0,13	12,35 ± 0,09	93	
	6	21,38 ± 0,17	12,79 ± 0,11	98	
	7	21,36 ± 0,12	12,84 ± 0,08	95	
	8	20,83 ± 0,13	13,50 ± 0,09	123	
	9	20,80 ± 0,11	13,52 ± 0,09	122	
	10	21,48 ± 0,13	*15,21 ± 0,11*	50	
	11	20,44 ± 0,09	13,58 ± 0,10	114	
	12	21,24 ± 0,12	13,18 ± 0,09	140	24 ±
	13	21,55 ± 0,15	*14,75 ± 0,18*	78	
	14	20,45 ± 0,12	13,24 ± 0,09	70	
	15	20,20 ± 0,13	13,29 ± 0,10	65	
	16	19,30 ± 0,10	13,63 ± 0,12	88	
	17	20,49 ± 0,11	13,11 ± 0,11	128	24
	18	20,32 ± 0,11	13,08 ± 0,09	130	
	20	20,89 ± 0,13	13,17 ± 0,11	65	24
	21	20,19 ± 0,08	13,10 ± 0,08	104	
	22	20,45 ± 0,12	13,12 ± 0,10	87	
	23	*23,27 ± 0,11*	*15,56 ± 0,17*	110	
	24	20,91 ± 0,13	13,58 ± 0,09	121	
	25	20,12 ± 0,10	13,24 ± 0,10	60	
	26	19,91 ± 0,11	13,08 ± 0,11	35	
	27	*22,30 ± 0,16*	*15,06 ± 0,11*	66	
	28	20,55 ± 0,16	13,18 ± 0,09	90	
	29	19,62 ± 0,08	13,84 ± 0,11	48	
	30	*22,90 ± 0,13*	*15,10 ± 0,10*	122	
	31	19,40 ± 0,10	13,17 ± 0,12	95	
	34	21,78 ± 0,12	13,29 ± 0,06	130	24
	35	20,55 ± 0,12	13,58 ± 0,11	30	
	36	19,97 ± 0,11	13,35 ± 0,11	122	
	37	20,29 ± 0,12	13,00 ± 0,12	98	
	38	20,77 ± 0,15	13,09 ± 0,11	47	
	39	21,31 ± 0,14	13,85 ± 0,12	103	24
	40	21,18 ± 0,12	13,70 ± 0,10	137	24
	41	19,58 ± 0,10	13,49 ± 0,11	63	
	43	20,90 ± 0,16	13,22 ± 0,13	64	
	46	20,94 ± 0,18	13,67 ± 0,12	112	24
	47	20,88 ± 0,13	13,88 ± 0,11	125	24
	48	21,16 ± 0,11	12,68 ± 0,10	126	24

	Stomatal length	Stomatal breadth	Plant height in mm.	2*n*
Plant No. 49	21,73 ± 0,17	13,75 ± 0,13	110	24
50	20,41 ± 0,12	14,12 ± 0,09	118	
51	20,05 ± 0,11	13,57 ± 0,11	102	
52	21,09 ± 0,12	13,85 ± 0,11	102	
53	20,80 ± 0,12	14,27 ± 0,09	110	24 ±
54	20,25 ± 0,10	13,99 ± 0,10	142	24 ±
55	21,02 ± 0,12	13,23 ± 0,10	85	
56	20,28 ± 0,12	13,30 ± 0,10	120	24 ±
58	20,90 ± 0,13	13,60 ± 0,10	87	
59	20,89 ± 0,12	13,34 ± 0,09	96	24 + 2ff.
60	20,69 ± 0,17	*14,46 ± 0,15*	76	
62	*22,33 ± 0,14*	*15,75 ± 0,15*	80	
63	20,26 ± 0,11	13,20 ± 0,10	140	24
64	21,54 ± 0,11	15,06 ± 0,21	110	
65	21,15 ± 0,13	13,25 ± 0,10	75	
66	20,88 ± 0,12	13,65 ± 0,09	93	
67	20,88 ± 0,11	13,58 ± 0,10	111	
68	20,69 ± 0,11	13,72 ± 0,09	118	
69	20,70 ± 0,09	12,87 ± 0,11	50	
70	20,00 ± 0,08	13,49 ± 0,09	80	

caused by the genotypic constitution. The majority of the plants, however, have a normal growth habit and show ordinary growth in height. All the progeny-plants were root-fixed during the summer of 1945 on five different occasions. On account of the nature of the chromosomes it has unfortunately only been possible to count a few plants.

In *Picea Abies* the chromosomes are long and often lie entangled in the somatic cells during metaphase, with the result that it is usually impossible to determine the somatic number. Similar difficulties have been experienced by K. and H. J. Sax (1933) in attempts to determine the chromosome number in root-tips of Conifers.

Among the progeny of No. 181 it has been possible to count the chromosomes of 15 plants. Four of these counts are uncertain, however. Annoyingly enough, the chromosome number of some of the habitually deviating plants have not admitted of being counted, as will be seen from Table 3. In one of the plants with an ordinary growth-habit and normal stoma-size one plate has 24 chromosomes + two fragments (Fig. 34). Other plants counted have 24 chromosomes.

At the examination of the stomata in the progeny 35 cells were measured on each of three needles per plant. The needles were taken from the top of the plants. With the exception of two plants, in which 100 stomata were measured, 105 observations were accordingly made

per plant. Thus, altogether 6.500 measurements were made. On an average taken over the whole progeny the length of the stomata is $27{,}78 \pm 0{,}0023$ scale divisions and the corresponding cell-breadth $13{,}57 \pm 0{,}0016$ divisions (1 division $= 2{,}47\ \mu$). The variation in cell-size of the individual plants is given in Table 3. Four plants deviate very much in both breadth and length of cell and three plants in cell-breadth. In these plants the length and breadth of the cells are on an average 9,2 and 12,1 % larger than the corresponding mean of all the progeny of No. 181.

TABLE 4. *Analysis of variance. Length of stomata in progeny obtained from spruce 181 after free flowering.*

Variation	Df.	Sum of squares	Mean square	Quot.
Between plants	61	12.494,69	204,83	124,29***
Within plants (= error)	6438	10.610,11	1,648	$P < 0{,}001$
Total	6499	23.104,80		
Between needles within plants	124	1.363,66	11,0	6,67***
Within needles	6314	9.246,45	1,470	$P < 0{,}001$
Total (within plants)	6438	10.610,11		
Stomatal breadth in same progeny.				
Between plants	61	3.360,27	55,09	45,23***
Within plants (= error)	6438	7.840,53	1,218	$P < 0{,}001$
Total	6499	11,200,80		
Between needles within plants	124	1.392,75	11,23	9,22***
Within needles	6314	6.447,78	1,021	$P < 0{,}001$
Total (within plants)	6438	7.840,53		

In Table 4 the variation in stoma-size has been divided into *variation between plants* and *between needles within plants*. The table shows that there is a significant difference in cell-size between plants as well as between needles within plants. It is surprising that the last-mentioned variation is significant, since, as already pointed out, the needles were taken from the top of the plants.

VIII. CONCLUSIONS AND DISCUSSION.

With the exception of *Sequoia sempervirens*, *Pseudolarix amabilis* and *Juniperus chinensis Pfitzeriana* no stabilized polyploid species have been found among Pinaceae and Cupressaceae. These three species all have the tetraploid chromosome number and thus constitute the only cases within these plant-groups in which polyploids and environment have formed a favourable combination. Probably polyploid individuals occur now and then in other allied genera, although they have been unable to survive as stabilized species. It is of some interest to note that the three polyploid species found among coniferous trees seem to be autopolyploids (cf. K. and H. J. SAX, 1933). In nature no allopolyploid species or individuals of spontaneous origin are at present known among the Conifers, although SYRACH LARSEN and WESTERGAARD (1938), by crossing *Larix decidua* ($2n = 24$) to *L. occidentalis* ($2n = 24$), succeeded in obtaining a triploid hybrid ($2n = 36$) which, since two different species are concerned here, must be considered to be an allotriploid.

The autotetraploids found among the Conifers would seem to have appeared in two steps, probably through an unreduced ovum in a diploid tree being fertilized by a haploid pollen-grain from another diploid tree and in course of time giving rise to a triploid plant. This triploid in its turn probably produced an unreduced egg-cell that was fertilized by a haploid male gamete. If the tetraploids had arisen in one step, this would imply that an unreduced macrospore had been fertilized by an unreduced microspore which had likewise arisen. However, the chance of two unreduced gametes arising and fertilizing each other must be said to be infinitely small. Moreover, a fertilization of that kind would probably cause a disturbed development of the seeds, as during seed development three different tissues are in very intimate connection with each other, viz. the investing somatic tissues of the mother-tree and the embryo and endosperm tissues (cf. MÜNTZING, 1933, 1936).

After normal fusion of haploid gametes in diploid Conifers the relation between the chromosome numbers of these tissues is expressed by the ratio 2 : 2 : 1, in the order given. If it were possible for two unreduced gametes in diploid coniferous trees to fuse with each other, the corresponding ratio would be 2 : 4 : 2, which may cause physiological as well as morphological disturbances in the connection between embryo- and endosperm-tissues and the surrounding diploid tissues. It is of course also conceivable that triploid plants may arise as a result of a haploid egg-cell being fertilized by an unreduced pollen-grain. That

seems in fact to have been the case in the triploid larch hybrid obtained (cf. SYRACH LARSEN and WESTERGAARD, 1938). Among the Angiosperms, however, in crosses between diploid individuals, triploid progeny seem more likely to arise when unreduced egg-cells are fertilized by haploid pollen-grains than in the case in the other direction. These differences in seed development depend, according to MÜNTZING, in all probability on numerical changes in the chromosome sets as between embryo, endosperm and the surrounding somatic tissues of the maternal plant (cf. MÜNTZING, 1933, 1936).

The relation in chromosome numbers between the endosperm and the investing somatic tissues of the mother-plant frequently seems to be of importance for the development of the endosperm. Probably, however, different species react differently to disturbances between these tissues. The first-mentioned conclusion corresponds well with the results reported by COOPER and BRINK (1940) respecting the development of the endosperm and the innermost cell-layer of the integument after self-fertilization in *Nicotiana rustica* and after the cross *N. rustica* ($n = 24$) $\times$ *N. glutinosa* ($n = 12$). In that case the endosperm and investing tissue develop quite differently after self-fertilization and crossing. After selfing the endosperm and its surrounding cell-layers grow normally (about 77 % of the volume of the ovule consists of endosperm and 23 % of »nucellus»), while the relation between these tissues in the hybrid *N. rustica* $\times$ *N. glutinosa* is essentially changed (only about 25 % is endosperm while 75 % of the volume of the ovule consists of »nucellus»). In the last-mentioned case the innermost cell-layer of the inner integument produces a considerable number of cells, which in course of time completely enclose the endosperm. This results in a rupture of the funicle, and communication between endosperm and placenta ceases. This type of abortion may be associated with the change in the chromosome relations between endosperm and surrounding somatic tissue. At inbreeding the chromosome ratio as between embryo, endosperm and surrounding somatic tissue is the normal, 2 : 3 : 2, or in this case 4 : 6 : 4, while the ratio as between the same tissues after the cross mentioned is 3 : 5 : 4.

Some abnormal conditions in the development of endosperm and antipodal cells after crossing *Hordeum jubatum* ($n = 14$) to *Secale cereale* ($n = 7$) have been described by BRINK and COOPER (1944) and COOPER and BRINK (1944). These disturbances, however, may also depend on other causes than solely changes in the chromosome sets between neighbouring tissues.

Besides the cases already mentioned, polyploid forms may also occur as a result of asyndesis. This implies nothing fundamentally new in comparison with the cases just described, as in that case, too, the polyploids must be considered to arise through the union of an unreduced and a haploid gamete or of two unreduced gametes. However, the possibility of unreduced gametes appearing is considerably greater in asyndetic trees than in trees having a completely normal reduction division. It cannot therefore be ruled out that a number of triploid plants may appear in the vicinity of an asyndetic tree. These triploids will then have many opportunities of crossing either with neighbouring diploids or with triploids and aneuploid half-sibling plants in the vicinity. Such crosses ought to result in the production of, for instance, tetraploids and new triploids.

Although the Conifers undoubtedly suffer from self-sterility, it has been shown that a certain amount of seed-setting may be expected after self-fertilization of, e. g., spruce and pine (cf. DENGLER, 1932, 1939). The polyploids that arise, like other polyploids among the Angiosperms, should be more self-fertile and less susceptible to inbreeding depression than the corresponding diploid Conifers. In the spruce, however, some degree of metandry (protogony) seems to exist (SYLVÉN, 1910; SYRACH LARSEN, 1937). This difference in flowering-times of female and male flowers on the same tree may therefore completely or partially prevent self-fertilization. Consequently, cross-fertilization may be considered the normal and probably the only possible means of fecundation at wind-pollination of the spruce. If the incomplete or completely absent chromosome pairing during meiosis is conditioned by genes, single asyndetic individuals ought to be able to »segregate out» continuously in the populations.

Certainly it is probable that a large number of the progeny of an asyndetic tree are marked by disturbed meiosis and low pollen fertility and seed production, whereby the spread of the new polyploids is rendered more difficult. None the less, there have been possibilities for stabilized polyploid species to originate among our native Conifers as well, this not least of all owing to the fact that the Conifers belong to a demonstrably very old group of plants. There is accordingly reason to think that polyploids might also have arisen by a vegetative method. Tetraploid cells and sectors have been found in the ordinary diploid tissue of several plants, and such cells may also occur in the Conifers. Should these tetraploid sectors give rise to a branch with a collection of tetraploid inflorescences, a number of diploid gametes must thus be the result.

It is evident that changes in the chromosome numbers have been of little account in the differentiation of most coniferous genera. No stabilized polyploid species have so far been found among our most common Conifers, e. g., spruce, pine and larch. Thus, it is a plausible presumption that existing diploid Conifers are best adapted to present-day environmental conditions, and that as a rule their chromosome number may be considered to be that constituting the maximum of the species in question.

In spite of the absence of stabilized polyploid species among, for instance, *Picea* and *Pinus* solitary finds of triploid and tetraploid individuals would have been expected. No trees with morphological characters deviating from the normal type and suggestive of polyploidy have been found in middle-aged and older stands of Conifers in spite of numerous examinations having been made of, among others, the seed-setting in different coniferous trees. Hence the frequency of any possibly existing polyploid spruces with normal or more than normal growth capacity must be very low, or else polyploid zygotes within certain genera must have already died during the development of the embryo owing to disturbances in the chromosome-number relation between embryo, endosperm and surrounding somatic tissue. The polyploids may also have consisted of dwarfs or stunted trees, which have died or are cleared away at thinnings of the stands. That in all probability the frequency of polyploid Conifers is remarkably low may partly be due to the fact that the method of fertilization does not allow of polyploids or corresponding diploids being sexually isolated, or partly to the above-mentioned chromosomal disturbances between different embryonal tissues of the polyploid zygote. Changes in cell-size or in rate of cell-division may be quite sufficient to prevent normal seed development or to produce so-called somatoplastic sterility (cf. COOPER and BRINK, 1940, 1944).

As regards the dissemination of chromosome-altered forms, this must depend to a large extent on the mode of fertilization. Polyploid cross-fertilizers that are not isolated from original diploid types in the vicinity will soon, according to MÜNTZING (1933), be broken down through chromosome elimination during a series of back-crossings with the original diploid material. As different species react in different ways to chromosome doubling, it is not excluded that, for instance, polyploids of *Picea Abies* also have a vitality inferior to that of the corresponding diploids.

According to SAX's theory (cf. SAX, 1932), the absence among Coni-

fers of polyploids with a high number of interstitial chiasmata per bivalent may to some extent depend upon an irregular distribution of homologous chromosomes during meiosis in any autopolyploids that may arise. In *Picea Abies* the average number of chiasmata per bivalent is 2,7. Moreover, most of these chiasmata are interstitial. Hence it is not unlikely that chromosome pairing at meiosis in tetraploid spruces will result in a very intimate and general formation of quadrivalents. As a rule a quadrivalent formation of this kind would cause an irregular distribution of homologous chromosomes to the two poles, which might result in extreme gamete sterility.

The pollen fertility in *Pseudolarix* and the tetraploid *Juniperus chinensis Pfitzeriana*, however, affords no support for the hypothesis that quadrivalent configurations during meiosis would cause non-disjunction (cf. K. and H. J. SAX, 1933). The pollen sterility in *Pseudolarix* is less than 5 % and in the tetraploid *Juniperus* 6 %, which is lower than in the corresponding diploids. The number of chiasmata per bivalent is 2,1 in *Pseudolarix* and 2,2 in *Juniperus communis* ($2n = 22$).

The meiotic division in *Picea Abies* seems to be normal as a rule, though irregularities occur. Three »sticky trees» have been encountered. These observed cases of stickiness, however, may be of modificative nature and in that case caused by the great changes of temperatures between day and night. In addition to these anomalies one asyndetic spruce has been discovered. *This case of asyndesis seems to be the only one that has hitherto been observed among the Gymnosperms.*

Several cases of asynapsis are known within the Angiosperms and some in the animal kingdom, e. g., among *Drosophila pseudo-obscura*, *Pygaera* hybrids (cf. DARLINGTON, 1937, p. 414) and *Triton* (BÖÖK, 1945). A summary of previously examined instances of asynapsis has been presented by PRAKKEN (1943), which has been supplemented by JOHNSSON (1944). PRAKKEN distinguishes the following groups of synapsis: (1) Asynapsis due to the action of a distinct gene or genes (or some slight structural change). (2) Asynapsis caused by loss of a chromosome pair. (3) Asynapsis induced by external conditions. (4) Asynapsis as a normal process in apomictic organisms. (5) Asynapsis depending upon mechanical chromosome conditions (structure, number). (6) Asynapsis in species hybrids.

The instance of asyndesis described here in *Picea Abies* may in all probability be assigned to group 1. More or less certain cases of gene-conditioned asyndesis are known. Genetically governed asyndesis has been described in, among others, *Zea* (BEADLE and MCCLINTOCK, 1928; BEADLE, 1930, 1933), *Nicotiana tabacum* (CLAUSEN, 1931), *Hordeum* (EKSTRAND, 1932), *Datura* (BERGNER, CARTLEDGE and BLAKESLEE, 1934), *Nicotiana sylvestris* (GOODSPEED and AVERY, 1939), *Alopecurus myosuroides* (JOHNSSON, 1941, 1944), *Secale* (PRAKKEN, 1943), *Rumex* (LÖVE, 1943), and *Triticum vulgare* (LI, PAO and LI, 1945).

BEADLE has shown earlier that there are recessive genes which

cause asynapsis. In addition genes occur that induce supernumerary divisions in the gametophytes, and such as to some extent suppress cell-wall formation. CLARK (1940) even found a gene, in maize, that diverged the heterotypic spindle instead of converging it. The result of this will be that the chromosomes do not pass to the poles to form normal telophase nuclei but remain scattered in the cells and give rise to many micro-nuclei. This mono-factorial recessive asynapsis shows widely differing characteristics in different objects. The relative number of univalents, rod and ring bivalents, and chiasmata varies from case to case. Variation is likewise exhibited by the chromosome pairing during early prophase, the behaviour of the univalents during the heterotypical division, and the number of restitution nuclei and functional dyad pollen grains, etc. within different materials.

Complete pairing of chromosomes during pachytene (desynapsis) has been observed in individuals of *Zea mays* (BEADLE, 1930, 1933), *Crepis capillaris* (HOLLINGSHEAD, 1930; RICHARDSON, 1935), *Nicotiana sylvestris* (GOODSPEED and AVERY, 1939), triploid *Allium amplectens* (LEVAN, 1938, 1940), *Secale cereale* (PRAKKEN, 1943), *Triticum vulgare* (LI, PAO and LI, 1945), and *Picea Abies* (in the present work). On the other hand, no zygotene and pachytene pairing seems to take place in asynaptic *Rumex acetosa* (YAMAMOTO, 1934) and there is incomplete prophase pairing in, e. g., asynaptic dwarf fatoid oats with 40 chromosomes (HUSKINS and HEARNE, 1933) and probably in *Alopecurus myosuroides* (JOHNSSON, 1944).

In all asyndetic individuals showing more or less complete zygotene and pachytene pairing the separation of the paired chromosomes generally takes place gradually. The time at which this separation takes place, however, appears to vary in different asyndetic materials and even between individuals of the same species. In the above-described *Picea* instance the paired chromosomes already begin to fall apart at the end of pachytene. During the middle and late diplotene stage only a few bivalents occur, or about the same bivalent frequency as was stated for metaphase I.

The same is to some extent the case in maize (BEADLE, 1930, 1933) and wheat (LI, PAO and LI, 1945). According to the last-named Chinese scientists, the apparently paired chromosome threads in some desynaptic wheat plants separate at pachytene, while in other wheat plants the corresponding chromosome separation does not take place until the diplotene phase. In *Allium amplectens* (LEVAN, 1940) and *Secale cereale* (PRAKKEN, 1943) the paired members are kept together during diplotene by relational coiling, and even during diakinesis many pairs of univalents appear.

Several hypothesis have been advanced to explain the separation of the homologous chromosomes of asynaptic plants during prophase. According to DARLINGTON, the failure of chiasma formation may be due to the fact that at leptotene the chromosome separation is relatively far advanced, or that the beginning of prophase is relatively delayed. In both cases, accordingly, it is presumed that the chromosome threads have already parted during leptotene, which is considered to prevent a normal attraction of homologous chromosome threads. In partial asynapsis the peculiar situation must arise, according to DARLINGTON, that only some of the chromosomes divide lengthwise during leptotene, while other chromosome threads remain undivided.

HUSKINS and SMITH (cf. HUSKINS, 1932, p. 6), however, seem to have observed in *Fritillaria meleagris* that certain rather considerable portions of the chromosomes are longitudinally divided during the early heterotypic prophase, and that a complete pairing takes place between undivided homologous chromosome segments, while the corresponding split sections do not pair, which in itself ought to cause partial asynapsis. From that point, however, it is not a far step for the longitudinal division to take in the whole or the greater part of certain chromosomes, as at the time of pairing homologous chromosomes have probably not yet managed to find each other.

Another theory has been presented by SAX and SAX (1935). According to these scientists, one of the prerequisites for chromosome pairing is that the chromosome threads are despiralized before the zygotene phase begins, since a high degree of spiralization seems to prevent pairing. During a long prophase the chromosome threads therefore have greater possibilities of despiralizing before the beginning of the zygotene phase than during a short and very rapidly passing early prophase.

Thus, these two hypotheses, presupposing as they do that the chromosomes have been prevented from pairing during prophase, do not hold in all observed cases of desynapsis.

In the asyndetic spruce the behaviour of the univalents during metaphase I is of some interest, as it also is in the majority of asyndetic Angiosperms. When bivalents are altogether lacking in the pollen mother-cells, which is the case in one-third of the cells of the asyndetic spruce, the univalents preferably pass straight to the poles. This is inferable from the behaviour of the bivalents in other neighbouring cells during early metaphase I. *Thus, in cells with solely univalents the latter are distributed to the poles during metaphase I.* On the other hand, if there are one or more bivalents in a pollen mother-cell, a

greater or a smaller number of univalents are often drawn to the equator and are forced to range themselves in an equatorial plate in common with the bivalents.

The different behaviour of the univalents in these two cases seems to be explained by ÖSTERGREN's centromere theory (unpublished; cf. ÖSTERGREN and PRAKKEN, 1946). ÖSTERGREN presumes that at a somatic mitosis the chromosomes during metaphase have a centromere consisting of the sum of two anaphase-centromeres. This undivided centromere is equally attracted by both poles and therefore goes to the equator. When the centromere has divided, each of its halves is directed toward one pole only and is accordingly attracted solely by this pole. According to ÖSTERGREN, during metaphase I the univalents of, for instance, the asyndetic spruce will be in a state *between singleness and doubleness*. At the earliest period of metaphase I, when the centromeres of the univalents are incompletely polarized, the univalents migrate to one of the poles. On the other hand, when the centromeres of the univalents have become doubled or polarized the univalents pass to the equator.

Only a small number of bivalents occur in the asyndetic spruce. Judging from the length of the chromosomes and the positions of the centromeres, pairing between homologous chromosomes will as a rule have taken place among the bivalents. Still, we ought not to leave out of account the possibility of non-homologous pairing. LEVAN (1942, 1945) has found that the chromosome threads are distinctly paired during prophase in haploid rye and haploid sugar-beet. Non-homologous pairing has already been observed by MCCLINTOCK (1933) in maize. In addition LEVAN found several symmetric bivalents in his haploid material. The most interesting find in LEVAN's studies of rye and sugar-beet, however, is that almost all chiasmata are distributed at random or show good agreement with the POISSON's distribution, which shows that it cannot be a question of *solely chiasmata formed between duplicated chromosome segments*. From these results it seems bold — except in cases of complete pachytene pairing (or desynapsis) — to assume unreservedly that all observed bivalents of asyndetic plants are the products of homologous pairing.

As was earlier pointed out, a number of irregularities occur during the first division in the asyndetic spruce, such as irregular distribution of univalents, non-disjunction, premature centromere division or divided univalents, fragmentation, stickiness, and misdivision. Difficulties generally arise at the separation of chiasmata during anaphase I, and in

consequence a relatively high percentage of chromatin bridges occur in relation to the number of bivalents. These anomalies during the heterotypic division result in an almost equally irregular homotypic division, with chromosome elimination and greatly varying chromosome numbers in the gametes. As expected, this ultimately results in an abnormally high pollen sterility. Only two per cent morphologically good pollen have been produced. Still, this low percentage probably includes a few unreduced gametes that are capable of functioning. Judging from the seed-setting in the asyndetic spruce, we may venture to conclude that the embryo-sac mother-cells exhibit about the same degree of asyndesis as the pollen mother-cells.

Besides other methods at present being employed to produce polyploids in experimental breeding work on spruce, reciprocal crosses between this asyndetic spruce and normal spruces ought to be carried out to the greatest possible extent, it being conceivable that a polyploid may be obtained here and there as a result of such crossing experiments.

Finally, there may be reason to point out that no greater fundamental differences seem to exist between the above-reported case of asyndesis in a representative of the Gymnosperms and formerly known instances of the same cytological phenomenon within the Angiosperms.

SUMMARY.

(1) A case of asyndesis in *Picea Abies* has been observed. Comparisons have been made between meiotic divisions in normal spruces and the asyndetic specimen.

(2) Meiotic division seems to be very regular in spruces in general, though exceptions occur. During metaphase I the average number of chiasmata per bivalent is 2,7. No significant difference in chiasma frequency has been observed as between normal spruces. Most chiasmata have an interstitial position and do not terminalize at all or very inconsiderably.

(3) Microsporogenesis proceeds according to the simultaneous scheme in normal spruces and as a rule in the asyndetic spruce.

(4) The zygotene and pachytene pairing in the asyndetic spruce is almost complete.

(5) During metaphase I bivalents occur in the asyndetic spruce in 41 % of the number of cells, and the number of bivalents per cell varies between 1 and 8. In number the bivalents in these cells amount to 19,35 % of the number possible. In all examined pollen mother-cells

the frequency of bivalents is only 11,77 % of the number of possible bivalents. Of this frequency, 10,96 % consist of rod bivalents and 0,81 % of ring bivalents.

(6) Premature centromere division occurs during anaphase I in 4,1 % of the number of pollen mother-cells.

(7) Chromosome distribution to the poles is not altogether at random. The extreme groups 0 : 24, 1 : 23, 2 : 22 and so on are over-represented owing to the fact that the chromosomes show a tendency to appear in groups, which often behave as a unity.

(8) Stickiness has been observed in a number of pollen mother-cells in the asyndetic spruce.

(9) During interkinesis the cells exhibit a varying number of nuclei. In one case a cell with 16 nuclei was observed. Restitution nuclei have been formed in 8,76 % of the number of cells.

(10) The second division as well as the first division are very irregular owing to elimination of chromatids, stickiness and irregular chromosome distribution.

(11) The »tetrads» often contain micro-nuclei, and the morphological pollen fertility is very poor.

(12) Besides being observed in the asyndetic spruce, stickiness has been found in pollen mother-cells of three other spruces.

(13) Progeny obtained from the asyndetic spruce after free flowering show varying height growth, growth habit and stomatal size.

(14) The sparse occurrence of stabilized polyploid species within the Conifers has been discussed as well as various reasons for the fact that no single polyploid tree has been found within *Picea Abies*.

Lund, March 5th, 1946.

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"GENE DIVISIBILITY", AS STUDIED BY DIFFERENCES IN BAR FACET NUMBERS IN DROSOPHILA MELANOGASTER

BY *G. BONNIER, B. RASMUSON* AND *M. RASMUSON*
ANIMAL BREEDING INSTITUTE, WIAD, ELDTOMTA, AND UNIVERSITY OF STOCKHOLM, SWEDEN

I. THE PROBLEM.

THE present article is a continuation of studies on the effect of the gene »Exaggeration of Bar» (symbol *Eb*) on which an earlier report has been given (BONNIER, NORDENSKIÖLD and BÅGMAN, 1943). The principal facts concerning this gene which were brought together in this report were the following. *Eb* is a dominant sex-linked lethal which reduces crossing-over between Bar und fused from 2,5 to about 1,5 units. Linkage tests show that *Eb* is situated close to the right of Bar, at a map distance of probably less than 0,1 units. In co-operation with Bar it intensifies the Bar effect. A *B*/*Eb* female has eyes of the homozygous Bar size, and a *B Eb*/+ female has still narrower eyes. *Eb* does thus also show the Bar position effect. When no Bar gene is present an *Eb*/+ female has round eyes, but together with Bar it acts as though it were a (lethal) Bar gene. The exact origin of *Eb* is not known with certainty. But the data show that it must have occurred in a female with attached *X*'s homozygous with respect to Bar. As females *B*/*B* and *B*/*Eb* are similar in appearance (at least as long as no facet counts are made), it is quite natural that the true moment of occurrence of *Eb* escaped attention. It was in connection with detachments that it was first observed that something had gone wrong. Such detachments did show that they no longer contained *B* but the new gene *Eb* instead, and it was first believed that *Eb* was an allele of *B*. Later, however, cross-overs between *B* and *Eb* were found, but the crossing-over percentage was of the same order of magnitude as the percentage of unequal crossing-over within the Bar region. The salivary chromosomes of females *Eb*/+ were found to contain only one of the two segments of Bar. These chromosomes indicated furthermore a slight possibility that an *Eb* chromosome is deficient of one of the faint bands of the Bar segment.

It may thus be concluded that *Eb* occurred as a (spontaneous) mutation from Bar, and that the Bar chromosome from which it originated lost one of its segments probably in connection with the mutation. But the most interesting fact was revealed when the facet counts were made. As it is known that facet number modifiers are very common,

TABLE 1. *Excerpt from Table 11 in* BONNIER, NORDENSKIÖLD *and* BÅGMAN (*1943*). *The number of the strains* (*leftmost column*) *does not refer to Table 11, but are given as references to the present paper only. The genotypes of the female show only the genes B and Eb and not other genes* (*see text*).

Strain No.	Genotype of females	Number of eyes counted	Mean facet number
1	*B/Eb*	107	84,3
2	*BEb/+*	190	40,6
3	*B/Eb*	102	90,2
4	*B/Eb*	116	89,8

the counts were preceded by standardizing isogeneous lines by a procedure of inbreeding (v. i.). When such lines had been established, search was again made for cross-overs between *B* and *Eb* (using *f* and *fu* as marker genes). Thus there were, among others, four different strains in which the females were constituted as follows (disregarding all genes other than *B* and *Eb*): (1) *B/Eb*; (2) *B Eb/+*, which occurred through crossing-over in strain 1; (3) and (4) *B/Eb*, which occurred through crossing-over in strain 2 on two different occasions. The facet counts in these four strains gave the results shown in Table 1. (For further details cf. Table 11 in BONNIER, NORDENSKIÖLD and BÅGMAN, 1943.) From an analysis of variance with regard to the comparison between the three strains of *B/Eb* females it was found that whereas there was no statistical difference between the facet numbers of the two recovered strains 3 and 4, 90,2 and 89,8 respectively, the difference between these two on the one hand, and the original strain No. 1 with its 84,3 facets on the other, was statistically very significant. The results did thus show that two lines of *B/Eb* females of which the one hand had originated from the other through two instances of crossing-over between *B* and *Eb*, but which otherwise must have been isogeneous (or at least were intended to be isogeneous), had different numbers of facets. It seemed then necessary to try to elucidate somewhat further

this very peculiar situation. The present article contains a description of the new experiments.

II. THE PROCEDURE OF STANDARDIZING ISOGENEOUS STRAINS.

The principles of the procedure by which standardized isogeneous strains were synthesized has been put together in the preceding article (BONNIER, NORDENSKIÖLD and BÅGMAN, 1943). As it is, however, of importance to be in a position to judge — if possible — how far the isogeneity really reaches, all known details concerning the relevant strains will be given here. Unfortunately the exact number of inbreeding generations was checked only during the beginning of the standardizing procedures, and consequently only minimum numbers can be given.

Two different kinds of procedure were performed, viz. standardizing the *X*-chromosomes and making the autosomes homozygous.

The X-chromosome. — The *X*'s were standardized by introducing the genes *sc cv v car*. The stock-cultures used to synthesize strains relevant to the present article were

f B Eb/+
sc cv v f
sc cv v f car
f B
f fu

The males in some of the stocks (e. g., *f fu*) were kept going by crossing to *y* attached *X* females. The *sc cv v f car* stock had at an earlier instance been made by crossings between flies from the *sc cv v f* stock and a stock with *car*. The standardization procedure for the females was as follows: *f B Eb*/+ × *sc cv v f car*; *f B Eb*/*sc cv v f car* × *sc cv v f car*; *sc cv v f B Eb*/*sc cv v f car* × *sc cv v f car*; *sc cv v f B Eb car*/*sc cv v f car* × *sc cv v f car*.

Males *f B*: *sc cv v f*/*sc cv v f* × *f B*; *sc cv v f*/*f B* × *sc cv v f*; *sc cv v f B*/*sc cv v f* × *sc cv v f car*; *sc cv v f B*/*sc cv v f car* × *sc cv v f car*; *sc cv v f B car*/*sc cv v f car* × *sc cv v f B car*; *y* attached *X* × *sc cv v f B car* (»series B»; vide infra).

Males *f fu*: *sc cv v f car*/*sc cv v f car* × *f fu*; *sc cv v f car*/*f f u* × *sc cv v f car*; *sc cv v f car*/*sc cv v f car* × *sc cv v f fu*; *sc cv v f car*/*sc cv v f*

fu × + (from strain »hom.+»; vide infra); *y* attached *X* × *sc cv v f fu car* (»series B»; vide infra).

The autosomes. — The autosomes were made homozygous by means of a number of inbreeding procedures (Fig. 1).

First a wild type strain was propagated by brother—sister matings in pair-cultures. This strain was called »hom.+». Secondly *y* females with attached *X*'s were crossed with males from hom.+ of the 12th (or perhaps of a still later) inbreeding generation. This constitutes

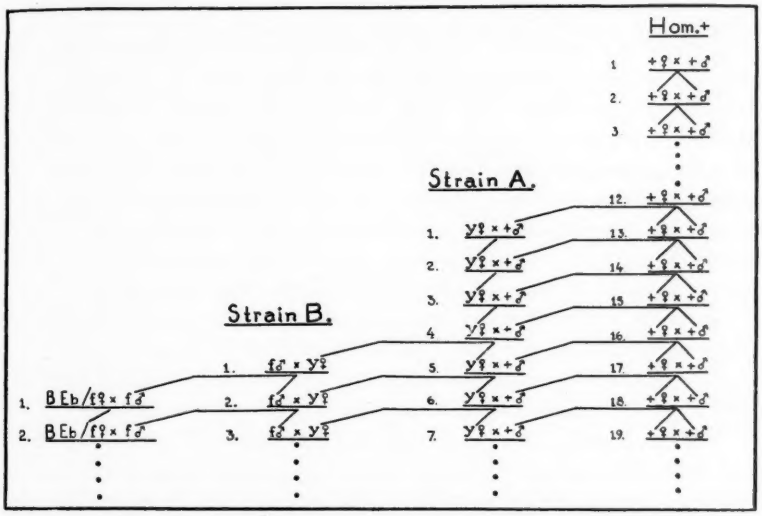

Fig. 1. Pedigree of lines Hom.+, Strain A, Strain B and *B Eb* (cf. text).

»series A». In every new generation of A, *y* females were crossed to +males from later generations of series hom.+. Simultaneously *y* females from series A were crossed to *X*-chromosome standardized males with *f*, *f B*, and *f fu*. These series constitute the different »series B». In the case of *f* males, for instance, these were crossed to *y* females of the fifth generation of series A, and *f* sons (generation 2 of »series B with *f*») were crossed to *y* females of a new generation of series A. It was not always possible to keep the generations of series A and series B exactly timed, with the effect that the generation number of the males from »series B with *f*» did not remain exactly 5 short of the generation number of the females taken from series A during the whole procedure. Parallel with these crosses females *f B Eb/f* (with standardized *X*'s)

were crossed in the same way with males of the »series B with *f*». The first generation of females *f B Eb/f* was crossed with *f* females of this B series of generation 2. Fig. 1 shows how the different crosses were chained to each other. Series B with *f B* and with *f fu* were also made up in the same way as »series B with *f*».

The following data give an idea of the number of generations within this procedure of making the autosomes homozygous.

The first generation of *y* attached females, series A, was crossed with wild males of the 12th (or perhaps a still later) generation of hom.+.

»Series B with *f*» was begun by crossing an *f* (*X* standardized) male with a *y* attached *X* female from the 5th generation of series A. The last cross in this series was made on Sept. 18, 1942, by crossing a *y* attached *X* female of the 23rd generation of series A with an *f* male of the 15th generation of »series B with *f*». Thereafter the chain was broken and the crosses *y* attached $X \times f$ (*X* standardized) were performed by inbreeding. Usually 2 females and 3 males from a single culture bottle were used as parents for the next generation. No check was then made of the number of these inbreeding generations.

Females *f B Eb/f* of the first generation were crossed with males *f* (*X* standardized) of the 2nd generation of »series B with *f*». The last of these crosses was made on Sept. 26, 1942, with females of the 15th generation. Thereafter inbreeding was as in the above case.

»Series B with *f B*» was started by crossing an *f B* (*X* standardized) male with a *y* attached *X* female of the 6th generation of series A. The last cross was made on Sept. 24, 1942, between a female of the 23rd generation from series A and a male of the 14th generation of the »series B with *f B*». Thereafter inbreeding as in the above cases.

»Series B with *f fu*» was started by crossing an *f fu* (*X* standardized) male with a *y* attached *X* female of the 22nd generation of series A. The last cross was made on Sept. 29, 1942, between a female of the 24th generation of series A and a male of »series B with *f fu*» of the 3rd generation. Thereafter inbreeding as in the above cases. After breaking the chains (which was necessitated by shortage of staff; see Fig. 1) the different lines have been propagated by more or less close inbreeding. (In the series hom.+, for instance, this inbreeding has been performed as brother—sister matings and has now — Oct. 1946 — passed its 126th generation.)

When these preliminaries had been fulfilled, an *f B Eb/f* female was crossed with an *f fu* male, and thereafter *f B Eb/f fu* females were

mated generation after generation with *f fu* males by breeding two females to three males from a single culture bottle of the preceding generation. In this new line of crosses, which hereafter will be named the *original f B Eb/f fu* line, search was made for cross-over chromosomes carrying *Eb* but not *B*. Non-cross-over females have either round eyes and are forked and fused, or they have Bar eyes of the narrow type and are forked but not fused. Females with round eyes showing forked but not fused must be cross-overs between *B* and *fu*, and such females were accordingly tested by outcrossing with *f B* males (from »series B with *f B*»). If the females of the progeny had the ordinary heterozygous

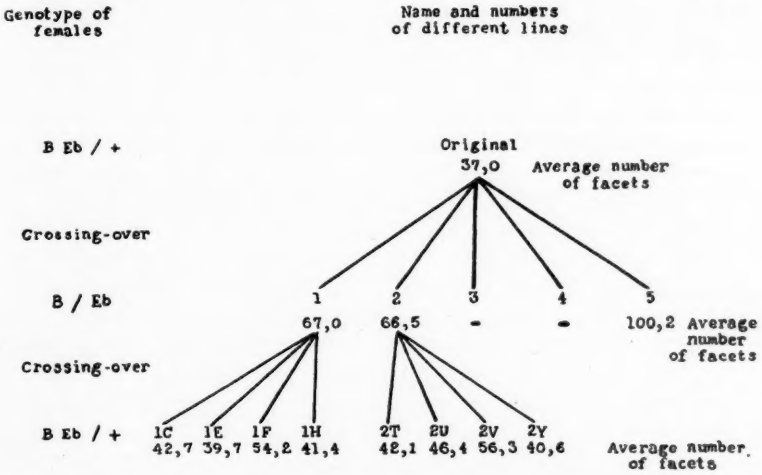

Fig. 2. Genealogy of the lines studied in the present paper. The genotypes of the females as given in the diagram show only the genes *B* and *Eb*. Apart from these genes a number of other genes were included in the genotypes (see text). The lines 1 C, 1 E, 1 F, 1 H, 2 T, 2 U, 2 V and 2 Y are in the text named »series», whereas the series 1 C, 1 E, 1 F, 1 H taken together, and the series 2 T, 2 U, 2 V, 2 Y taken together form two »groups», viz. group 1 and group 2. The line »original» is also in certain comparisons reckoned as a group.

Bar appearance, their mother could not be a carrier of *Eb*, and these cultures were consequently discarded. But if half of this female progeny showed the narrow Bar type the mother must have been an *f Eb/f fu* female. On five different occasions such females were found, and from each of these females a new line was started by the same kind of inbreeding and by alternating the crosses *f Eb/f fu* × *f B* and *f Eb/f B* × *f fu*. Before any facet counts were made, two of these lines

(viz. lines 3 and 4) were lost. Moreover, within these lines cross-overs between *B* and *Eb* were looked for in the progenies of the crosses *f Eb/f B* × *f fu*. Forked daughters which showed the narrow Bar condition, thus being of the constitution *f B Eb/f fu*, were crossed with *f fu* males, and this was repeated for each generation. Within strain 1 such cross-overs occurred on four different occasions, giving rise to the strains 1 C, 1 E, 1 F and 1 H. Likewise line 2 gave rise in a similar way to the four strains 2 T, 2 U, 2 V and 2 Y. No cross-overs were found within the lines 3, 4 and 5. Fig. 2 shows the descent of the different lines.

The facet counts comprised two different sets of lines, viz. those where the females (disregarding all genes except *B* and *Eb*) were of the constitution *Eb/B* (lines 1, 2 and 5), and those where the females had the constitution *B Eb/+* (lines »original», 1 C, 1 E, 1 F, 1 H, 2 T, 2 U, 2 V, 2 Y). It was the intention of the standardization procedure to make all lines of *Eb/B* and *B Eb/+* respectively exactly isogeneous, apart from differences within the region from *B* to *Eb*. Thus before going further in our description we must first consider in greater detail how far this procedure can be assumed to have gone.

The X-chromosome. — From the above description of the standardization of the *X*-chromosome it will be found that the »original» line of females *B Eb/+* might have been heterozygous for some parts within the region from *f* to *car*. But since the procedure by which the lines 1, 2, 3, 4 and 5, and from them the lines 1 C, 1 E, 1 F, 1 H, 2 T, 2 U, 2 V, 2 Y have been established, consists of crossing-over between *B* and *Eb*, all lines may be looked upon as quite isogeneous apart from differences within the *B—Eb* region.

The autosomes. — It will be found in the above description that the strain (Fig. 2) by which the »series B with *f fu*» was established had to be broken and substituted by inbreeding rather too early. In consequence, it cannot be claimed with certainty that »series B with *f fu*» was homozygous from the beginning. Neither can it be definitely claimed that its autosomes after further inbreeding were made isogeneous to the autosomes of »series B with *f B*», it being very much more certain that the latter series was already homozygous when first used. The first female of the »original» line — i. e. the female in the first cross *f B Eb/f fu* × *f fu* — may thus to a certain degree have been heterozygous in its autosomes. But the number of inbreeding generations (unfortunately not counted) which elapsed before the line 1 was established through crossing-over between *B* and *Eb* was certainly not

less than 20. However, as described above, the females of the lines 1, 2, 3, 4 and 5 were alternatively crossed to *f B* and *f fu* males, and since the autosomes of these two male lines may, as pointed out above, have been somewhat non-isogeneous, new heterozygozities may have been introduced. But there elapsed again a minimum of 20 generations from the establishment of line 1 to the establishment of lines 1 C, 1 E, 1 F and 1 H, and the same was the case from the establishment of line 2 to the establishment of the lines 2 T, 2 U, 2 V and 2 Y. Thus, though it is known that the speed with which the homozygotisation proceeds varies considerably in different lines (cf. BONNIER, 1947), it may be claimed reasonably and with a high degree of probability that at least the four lines 1 C, 1 E, 1 F, 1 H *inter se*, and the four lines 2 T, 2 U, 2 V, 2 Y *inter se*, were isogeneous — disregarding of course the differences within the *B—Eb* region.

III. ANALYSIS OF FACET COUNTS.

The different lines in which facet counts were to be made had been established at different instances. The facet counts were, however, made in cultures reared simultaneously, and therefore the number of generations which elapsed from the first establishment of a certain line until facet counts, were different for different lines. According to the working plan, it was hoped that there would have been available at least 4 lines with *B/Eb* females, and from each of these at least 4 lines with *B Eb/+* females. But on account of the small percentage of crossing-over between *B* and *Eb* this hope was never fulfilled. And as time passed, it was decided to perform the facet counts with the lines which were available. From one single culture bottle of each of the inbred lines a number of pair cultures were made up, and all these culture bottles from all the different lines were raised simultaneously in one single incubator at 25° C. In order to avoid overcrowding all bottles contained abundant food. Any other measures for the standardization of the environmental conditions, for instance as proposed by CHEVAIS (1942), were not taken. In order not to interfere with the temperature the incubator was kept closed until the 16th day after the start, when the cultures were emptied and all females preserved in alcohol. The left eves were dissected and the counts were made by the same technique (slightly modified) as described in the earlier paper (BONNIER, NORDENSKIÖLD and BÅGMAN, 1943). In Table 2 are the results summarized for all females of the constitution *B Eb/+*,

TABLE 2. *Facet counts of eyes from all flies of the constitution B Eb/+. The standard errors are computed by dividing the average mean square within cultures 28,2899 (see Table 4) by the number n and taking the square root, i. e. the number* $\frac{5,3188}{\sqrt{n}}$.

«Group»	«Series»	Culture	Number of left female eyes counted (n)	Average facet number
Original		1	23	39,83 ± 1,11
		2	31	39,81 ± 0,96
		3	21	40,10 ± 1,16
		4	9	38,67 ± 1,77
		5	8	38,75 ± 1,88
		6	40	30,70 ± 0,84
		Total	132	36,96 ± 0,46
1	1 C	1	37	48,27 ± 0,87
		2	45	44,24 ± 0,79
		3	26	43,54 ± 1,04
		4	33	45,30 ± 0,93
		5	54	38,46 ± 0,72
		6	28	40,36 ± 1,01
		7	40	51,55 ± 0,84
		8	70	40,71 ± 0,64
		9	39	34,44 ± 0,85
		Total	372	42,62 ± 0,28
	1 E	1	53	40,51 ± 0,73
		2	45	39,49 ± 0,79
		3	42	42,24 ± 0,82
		4	39	37,23 ± 0,85
		5	57	37,53 ± 0,70
		6	56	42,48 ± 0,71
		7	38	38,45 ± 0,86
		8	57	38.93 ± 0,70
		Total	387	39,66 ± 0,27
	1 F	1	7	54,29 ± 2,01
		2	4	56,25 ± 2,66
		3	8	53,13 ± 1,88
		Total	19	51,21 ± 1,22
	1 H	1	10	35,20 ± 1,68
		2	44	42,52 ± 0,80
		3	50	40,30 ± 0,75
		4	25	36,80 ± 1,06
		5	54	44,35 ± 0,72
		6	53	42,09 ± 0,73
		7	44	40,27 ± 0,80
		8	33	42,21 ± 0,93
		Total	313	41,37 ± 0,30
	Total 1		1091	41,42 ± 0,16
2	2 T	1	56	40,89 ± 0,71
		2	51	44,18 ± 0,74
		3	64	41,09 ± 0,66
		4	66	44,42 ± 0,65
		5	27	40,63 ± 1,02
		6	17	42,71 ± 1,29
		7	35	44,97 ± 0,90
		8	56	37,89 ± 0,71
		9	39	42,67 ± 0,85
		Total	411	42,06 ± 0,26
	2 U	1	52	45,67 ± 0,74
		2	31	45,36 ± 0,96
		3	54	46,57 ± 0,72
		4	54	49,78 ± 0,72
		5	14	47,43 ± 1,42
		6	29	41,55 ± 0,99
		7	44	47,91 ± 0,80
		8	46	42,22 ± 0,78
		9	54	49,04 ± 0,72
		Total	378	46,43 ± 0,29
	2 V	1	17	52,71 ± 1,29
		2	7	57,29 ± 2,01
		3	11	56,64 ± 1,60
		4	21	57,33 ± 1,16
		5	11	55,35 ± 1,60
		6	19	63,37 ± 1,22
		7	10	54,20 ± 1,68
		8	22	53,05 ± 1,13
		Total	118	56,32 ± 0,49
	2 Y	1	7	38,00 ± 2,01
		2	6	37,83 ± 2,17
		3	30	38,70 ± 0,97
		4	40	40,95 ± 0,84
		5	5	43,00 ± 2,38
		6	47	41,04 ± 0,78
		7	43	41,72 ± 0,81
		Total	178	40,62 ± 0,40
	Total 2		1085	44,90 ± 0,16
Total			2308	42,80 ± 0,11

TABLE 3. *Facet counts of eyes from all flies of the constitution B/Eb. The standard errors are computed by dividing the average mean square within cultures, 106,3514, by the number n and taking the square root, i. e. the number* $\frac{10,3127}{\sqrt{n}}$.

Line	Culture	Number of left female eyes counted (*n*)	Average facet number
1	1	58	$77,29 \pm 1,35$
	2	65	$70,92 \pm 1,28$
	3	17	$57,29 \pm 2,50$
	4	48	$75,94 \pm 1,49$
	5	55	$63,95 \pm 1,39$
	6	54	$65,56 \pm 1,40$
	7	49	$79,08 \pm 1,47$
	8	72	$64,08 \pm 1,22$
	9	28	$70,00 \pm 1,95$
	Total	446	$70,00 \pm 0,49$
2	1	48	$69,02 \pm 1,49$
	2	49	$64,08 \pm 1,47$
	3	28	$64,07 \pm 1,95$
	4	57	$65,68 \pm 1,37$
	5	42	$66,41 \pm 1,59$
	6	44	$63,48 \pm 1,55$
	7	36	$75,50 \pm 1,72$
	8	48	$66,15 \pm 1,49$
	9	59	$65,42 \pm 1,34$
	Total	411	$66,49 \pm 0,51$
5	1	20	$105,15 \pm 2,31$
	2	10	$91,80 \pm 3,26$
	3	25	$105,28 \pm 2,06$
	4	19	$92,58 \pm 2,37$
	Total	74	$100,16 \pm 1,20$
Total		931	$70,84 \pm 0,34$

and in Table 3 for the females of the constitution *B/Eb*. The standard errors for the average facet number in each culture are computed from the average mean square within cultures by taking its square root and dividing by the square root of the number of eyes. Thus, if this number

in a certain culture is n, the standard error is $\frac{5,3188}{\sqrt{n}}$ in Table 2, and $\frac{10,3127}{\sqrt{n}}$ in Table 3.

Females of the constitution B Eb/+. — Beginning with the results shown in Table 2, the following nomenclature will be used. All cultures belonging to one and the same line of the lines 1 C, 1 E, 1 F, 1 H, 2 T, 2 U, 2 V or 2 Y will be named a »series»; the four series 1 C, 1 E, 1 F and 1 H form a »group», and the same is the case with the four series 2 T, 2 U, 2 V and 2 Y as well as with the »original» line.

A glance at Table 3 shows that there possibly is a correlation between the number, n, of eyes counted in the different cultures and the average facet number, there seemingly being less facets the greater the value of n. This effect, if real, is certainly of an environmental nature and must be due to differential crowding. Now the number n is not exactly the same as the number of females which hatched out in the different cultures, because of the fact that the facets were not recorded on every female. The number of females were never counted, but it can certainly be claimed that the number n is — if not exactly so, at

TABLE 4. *Analysis of variance of the whole female material B Eb/+.*

Source of variation	Degrees of freedom	Mean squares
Between groups of cultures of different size	5	2193,8251
Within groups of cultures of equal size		
Between groups	8	1274,8670
Within groups		
Between series	18	1351,5746
Within series		
Between cultures	35	400,8894
Within cultures (error)	2241	28,2899
Total	2307	53,2835

Ratios $\frac{2193,8251}{1274,8670} = 1,72;\quad P > 0,2$

$\frac{1351,5746}{400,8894} = 3,37;\quad P < 0,001$

$\frac{400,8894}{28,2899} = 14,17;\quad P < 0,001$

TABLE 5. *Analysis of variance of females B Eb/+ belonging to »group» 1.*

Source of variation	Degrees of freedom	Mean squares	Symbols for reference to Table 7
Between groups of cultures of different size	4	449,6775	
Within groups of cultures of equal size		38,7713	*B*
Between series	8	848,6048	
Within series		32,7614	*b*
Between cultures	15	529,7725	
Within cultures	1063	25,7481	*w*
Total	1090	40,2792	*t*

Ratio $\frac{848{,}6048}{529{,}7725} = 1{,}60$; $P = 0{,}2$

TABLE 6. *Analysis of variance of females B Eb/+ belonging to »group» 2.*

Source of variation	Degrees of freedom	Mean squares	Symbols for reference to Table 7
Between groups of cultures of different size	5	1439,2861	
Within groups of cultures of equal size.....		52,6565	*B*
Between series	10	1762,0617	
Within series		36,6659	*b*
Between cultures	17	267,8997	
Within cultures	1052	32,9292	*w*
Total	1084	59,0524	*t*

Ratio $\frac{1762{,}0617}{267{,}8997} = 6{,}58$; $P < 0{,}001$

least with a very good approximation — proportional to the number of females hatched out. In the statistical analysis, therefore, in order to avoid the differential influence of the order of magnitude of n the cultures have first been grouped according to the value of n in 6 differ-

ent groups, viz. n = 4—10, 11—20, 21—30, 31—40, 41—50, 51—70. All comparisons have thereafter been made *within* these groups of n-values. The results of the computations are put together in Table 4, where the whole material of *B Eb*/+ eyes from Table 2 is analysed, and in Tables 5 and 6 where the results from groups 1 and 2 are dealt with separately. From these three tables a number of conclusions may be drawn.

(1) The mean square in Table 5 (group 1) corresponding to the four series averages is statistically not very much larger than the mean square corresponding to culture averages ($P > 0{,}2$). But in Table 6 (group 2) the first of these mean squares is significantly very much larger than the latter, and gives a mean square ratio of 6,58 which corresponds to P less than 0,001. This is by far the most interesting of the facts ascertained from Tables 4, 5 and 6. It might be objected that in group 2 (Table 6) there is an unusually small variation between the cultures, the mean square being only about 268, whereas in group 1 (Table 5) it is about 530. But even when the mean square between series from group 2 (Table 6), 1762,0617, is compared with the average mean square between cultures from groups 1 and 2, which is 390,6526 (not explicitly shown in the tables), a mean square ratio of 4,51 is found, and this (with 32 degrees of freedom) corresponds also to a P which is somewhat less than 0,001. Now, the four series within group 2 (as within group 1) were according to the preceding paragraph very probably isogeneous, apart from differences within the *B—Eb* region. The conclusion is that there must be genetical differences between the series belonging to group 2, and, granting the existence of the isogeneity, it is just those differences within this region which must be assumed responsible for the differences in facet numbers between the series means of group 2.

(2) As the mean square corresponding to group averages *within* n-values (Table 4) is only insignificantly less, and as the mean squares corresponding to series averages *within* n-values (Tables 5 and 6) are larger than the mean squares corresponding to the variations *between* different n-values, it is possible that the partition of the material according to the orders of magnitude of n was unnecessary. On the other hand, such a partition, even if it is not necessary, can cause no harm and will in no way interfere with the other conclusions to be made.

(3) As the mean squares corresponding to group averages (Table 4) is smaller than the mean square corresponding to series averages, the facet number averages of the three groups are statistically equal. The

same conclusion would have been drawn if only the two groups 1 and 2 had been compared.

(4) Within all series the mean squares corresponding to culture averages are very much larger than the mean squares corresponding to the variation within cultures, and consequently the environmental conditions prevailing within different culture bottles must have been effectively different in spite of the simultaneity of the raising of all cultures.

In this connection attention should be called to the fact that the partition of the material with respect to order of magnitude of n lowers the available degrees of freedom. The reason will be found obvious when it is mentioned that, for instance, for n lying between 11—20 only one group (group 2) is represented, and for n lying between 31—40 only one culture is represented within the series 1 H. It follows that if no such partition with respect to the n-value had been performed, not only would changed degrees of freedom have been found, but also changed significance values P. In this case the ratio between the mean squares corresponding to series and culture averages (within the series) had been, in the case of groups 1 and 2, 3,34 and 3,39 respectively. The corresponding degrees of freedom are: group 1, 3 and 24; group 2, 3 and 29 and thus for both groups P lies between 0,05 and 0,01. If both groups are pooled the ratio is 13,47 and P is less than 0,001. Thus whether the partition in question is performed or not, it must be concluded that statistically significant differences between means of series belonging to one and the same group do exist.

TABLE 7. *Subdivision of the total variance in facet numbers in B Eb/+ flies according to different causes of variation.*

Cause of variation	Formula for calculation (see Tables 5 and 6)	Percentage of total variance	
		Group 1	Group 2
Influence of size of culture	$\frac{t-B}{t}$	4	11
Differences between »series» within »groups» of cultures of equal size	$\frac{B-b}{t}$	15	27
Differences between cultures of equal size belonging to the same »series»	$\frac{b-w}{t}$	17	6
Differences between flies that hatched out within the same culture bottle	$\frac{w}{t}$	64	56

As it may be of some interest to know to what extent the total variation in facet numbers is due to the different sources of variation, estimates are made in Table 7 on the basis of the figures in Tables 5 and 6. It is seen that in group 1, as well as in group 2, the largest part of the variation is due to causes within the culture bottles. These causes cannot be analysed further, and are therefore as usual lumped together under the name of error. The largeness of this variation seems, however, to make it certain that the environmental conditions within the different bottles have been effectively different. In the case of group 2, where we have found the differences in series means to be highly significant, these differences are responsible for a variation, which next to error, is the largest one.

Females of the constitution B/Eb. — The counts within the lines 1, 2 and 5 of the constitution *B/Eb* were given in Table 3. To make a very thorough statistical analysis of these counts is of less importance, as their isogeneity is less certain. We therefore confine ourselves to making the remark that the line 5 has a facet average which differs considerably from the averages for lines 1 and 2. We believe that part of the cause of this difference is to be found in differences within the *B—Eb* region, but on account of the uncertain isogeneity, autosomal differences cannot be excluded.

IV. DISCUSSION AND CONCLUSIONS.

It has been shown in the preceding paragraph that facet number averages from *B Eb*/+ females belonging to different series within one and the same group are significantly different (at least in group 2). These differences cannot be assumed to be due to environmental causes. As has been pointed out, all cultures were raised simultaneously within the same incubator, and the environment can thus have a differential influence only in one of the following two ways: (1) on the individual fly within a certain culture bottle and/or (2) on the aggregate of the flies from the separate bottles. But in each case a »series» was made up of several bottles, and all bottles were mixed together in the incubator. It is thus extremely unlikely that environment can have influenced the series averages, and the differences between the series averages must be assumed to be due to genetical causes.

There are two possible kinds of genetical differences between the series within a group. (1) It may be remembered that each series originated from a single case of crossing-over between *B* and *Eb* in a

B/Eb female. The first kind of genetical difference, which must be assumed to occur, thus has its origin in different points of crossing-over within the *B—Eb* region at the moment the various series are founded. (2) The second cause is that in spite of the effects caused, the isogeneity of the different series — apart from the *B—Eb* region — was not complete, with the consequence that the series still differed with respect to genes affecting the facet number, but situated elsewhere.

As such efforts were taken to achieve isogeneity, it seems very improbable that differences due to modifying genes should still be possible. There is also an additional piece of evidence which, in its own way, speaks in favour of the isogeneity. The group which we have called »original» was directly, i. e. without any crossing-over, derived from the line of *B Eb/+* females described in the preceding article (BONNIER, NORDENSKIÖLD and BÅGMAN, 1943), and which is denoted as strain No. 2 in Table 1 of the present article. In this strain 190 eyes were counted and gave an average of 40,5 facets per eye. This average should therefore, if no further changes due to non-isogeneity had occurred, agree with the average of the »original» (Table 2), which is 37,0. An analysis of variance shows that the difference between the two averages 40,6 and 37,0 is insignificant. By making this analysis we cannot carry out a partition with regard to culture size, as no such partition was performed when the facets of the 190 eyes, strain 2 (Table 1), were counted. The mean square between the average of strain 2 (Table 1) and average of »original» (Table 2) is $1020{,}_{1925}$, and the mean square between the culture averages in »original» is $453{,}_{1750}$. The ratio of these two mean squares is 2,25, which, with the degrees of freedom 1 and 5 respectively, corresponds to a value for P not much less than 0,2. Now, as is seen from Table 2, »original» is made up of 6 cultures of which the last one differs considerably from the first 5. To make the same kind of analysis of variance after exclusion of the last culture would, however, not be fair, as those first 5 cultures are unusually uniform (as compared with the cultures within the other series). It seems most correct to compare the above mentioned mean square $1020{,}_{1925}$ with the mean square between averages of cultures belonging to one and the same series, and calculated from the whole material of Table 2. This last mean square is $739{,}_{8446}$, and the ratio of the two mean squares is 1,38, which, with the degrees of freedom 1 and 58, corresponds to a value of $P > 0{,}2$. It may finally be added that between the dates of the hatching out of the females of strain 2

(Table 1) and the females of »original» (Table 2) there had elapsed at least 75 generations.

It can never be stated if and when homozygosity is complete after a limited number of inbreeding generations even if this number is very large. Although it is thus impossible to prove decisively that the place of crossing-over is the *only* genetical determining factor in the variation of facet number in different »series» belonging to the same »group» it has, however, been proved as highly probable. Now, as mentioned above, *Eb* occurred in a Bar chromosome. But an *Eb* non-Bar chromosome contains only one of the two Bar segments. Furthermore, *Eb* has, when Bar is present, an effect of the same kind as Bar. From these considerations it was tentatively proposed in the preceding article (BONNIER, NORDENSKIÖLD and BÅGMAN, 1943) that *Eb* is in itself a part of Bar. In this article it was also pointed out that the fact that, by ordinary linkage tests, *Eb* is found to be situated to the right of Bar, may be explained by supposing that *Eb* lies within the Bar segment but so close to its right border that crossing-over between *Eb* and this right border has never occurred. But as crossing-over in different points within the *B—Eb* region gives different results, with regard to facet number, we must conclude that *Eb* has a linear dimension and that it is consequently not a »point» gene, but occupies a certain portion of the chromosome, which conforms with the crossing-over reducing effect of *Eb*. If this portion is not too short it is conceivable that one could find crossing-over cases giving the inverted sequence *Eb B* instead of the sequence *B Eb*. Concerning the position effect of *Eb*, it should indicate that the functioning of the *Eb* portion of the chromosome and its final effect on facet number depends upon how much of this portion lies in the one, and how much in the other *X*-chromosome. Thus, if the *Eb* portion is made up of two parts *Eb′* and *Eb″*, both genotypes *B Eb*/+ and *B*/*Eb* should more correctly be symbolized as *B Eb′*/*Eb″*. And the phenotype would then be dependent upon the relative lengths of the two parts *Eb′* and *Eb″*.

In this connection it is worth recalling that SUTTON (1943), in her studies on translocations within the Bar region, found Bar effects of different strengths. The phenotypic effects of her different translocations thus showed themselves as different numbers of facets.

As an alternative to the supposition that *Eb* is divided in portions *Eb′* and *Eb″*, it may be thought that it is the gene Bar itself which has been divided differently in the different series of our experiment. If this is so, it would be possible to find similar results in experiments with

Bar even in cases when *Eb* is not present. In new experiments, therefore, an attempt will be made to determine whether changes from *BB* to *B* and from *B* back to *BB* will lead to different numbers of facets in different series. But as the great obstacle to making conclusive proofs is the impossibility of being quite certain of the isogeneity when this is striven after by a procedure of homozygotisation, an attempt will be made to preserve isogeneity in these experiments by permanent heterozygosity.

Granting that the results which we have described in the present paper are not due to non-isogeneity, a certain kind of »gene divisibility» has been demonstrated. The possibility of such a divisibility has been discussed earlier in connection with the differential effects of scute inversions by RAFFEL and MULLER (1940). Our findings may possibly also support GOLDSCHMIDT's theories on the chromosome and the hereditary units (cf. GOLDSCHMIDT, 1946).

SUMMARY.

(1) In a preceding article (BONNIER, NORDENSKIÖLD and BÅGMAN, 1943) the sex-linked lethal gene »Exaggeration of Bar» with the symbol *Eb* was described. This gene occurred spontaneously in a Bar chromosome, but the *Eb* chromosome has only one of the duplicated Bar segments. By ordinary linkage tests *Eb* was localized as lying very close to the right of Bar. It reduces crossing-over between *B* and *fu* from 2,5 to about 1,5 units. A female heterozygous with respect to *Eb*, but not containing *B*, has round eyes; but when *B* is present *Eb* has the effect of a *B* gene. Thus, for instance, a female heterozygous with respect both to *B* and *Eb* has eyes of the homozygous Bar type. *Eb* shows also the typical Bar position effect inasmuch as *B Eb*/+ females have narrower eyes than *B*/*Eb* females. It was also tentatively proposed that *Eb* is situated within the Bar region.

(2) From a strain of *B Eb*/+ females, in the present article named »original», *B*/*Eb* females occurred through crossings-over on 5 different occasions, and, from each of these, strains of *B*/*Eb* females were established. (Later, however, two of these were lost.) From two of these strains again *B Eb*/+ females occurred through crossings-over at four different instances in each of the two *B*/*Eb* strains. In this way 2 »groups» of *B Eb*/+ strains were established, each containing 4 »series» (see Fig. 2). From all the different strains a number of cultures were raised simultaneously in the same incubator at 25° C, and the

number of facets in the left eyes of the female progeny were recorded.

(3) Prior to this facet counting an effort was made to make all *B Eb*/+ strains isogeneous through a procedure of marking the *X*-chromosome by known recessives and by making the autosomes homozygous by inbreeding. Such isogeneity can never be conclusively proved, but the manner in which the different series were established shows very probably that at least all series belonging to one and the same group are isogeneous. It is also shown that there are statistically significant differences between the facet numbers of flies belonging to different series, and that these differences cannot be caused by differential effects of environment.

(4) It is concluded that *Eb* — or perhaps the gene Bar itself — is »divisible» into parts, and that it is the lengths and interactions of these parts which are responsible for the determination of the facet number.

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EINE PERIKLINALCHIMÄRE IN DER GATTUNG SYRINGA

VON *HAKON HJELMQVIST*
BOTANISCHES INSTITUT, SYST. ABTEIL., LUND

(With a Summary in English)

ALS ich vor einigen Jahren mit einer Untersuchung über verschiedene Periklinalchimären beschäftigt war, erhielt ich bei einer Gelegenheit Mitteilung über einen Fliederstrauch, der einst einen Zweig mit ganz abweichender Blütenfarbe getragen hatte. Dieser Zweig war wieder verschwunden und da ich an anderen Exemplaren derselben Fliedersorte keine abweichenden Blüten finden konnte, hielt ich es für wahrscheinlich, dass es sich um eine Farbenmutation gehandelt hatte. Später gefundene Literaturangaben, besonders von ALEXANDER BRAUN (1873, 1874), der seinerzeit diese Fliedersorte eingehend studierte, wie auch weitere eigene Beobachtungen haben jedoch meine Ansicht ganz geändert; ohne Zweifel handelt es sich hier um eine Periklinalchimäre von interessanter Zusammensetzung.

Die *Syringa*-Form, um die es sich handelt, ist eine alte, heutzutage nicht oft angepflanzte Sorte, die unter dem Namen *Syringa chinensis* WILLD. var. *alba* (KIRCHN.) REHD. geht. In der Blattform stimmt sie mit *Syringa chinensis* überein. Die Blüten sind von abweichender Farbe; sie sind indessen nicht ganz weiss, wie der Name vermuten liesse, sondern blass lilafarbig; besonders an der Innenseite der Kronröhre und um ihre Mündung herum tritt der lila Anflug zutage; die Lappen des Saumes sind sehr schwach gefärbt, beinahe weiss. Interessant ist aber, dass die Blüten auch in der Form von *Syringa chinensis* abweichen und bestimmte Ähnlichkeiten mit *S. vulgaris* aufweisen, wie schon von BRAUN hervorgehoben ist. Die Lappen des Saumes sind nicht so ausgebreitet wie bei *S. chinensis*, sondern haben eingerollte Ränder, wie dies bei *S. vulgaris* der Fall ist, sodass sie schmäler und oben abgerundet aussehen, während sie bei *S. chinensis* im Verhältnis zur Länge breiter und kurz gespitzt erscheinen (siehe Fig. 1). Der Kelch, der bei *S. chinensis* rötlich ist und ziemlich lange Zähne hat, die durch spitzwinklige Einschnitte getrennt sind, ist hier wie bei *S. vulgaris* grün und hat breite, abgerundete Einkerbungen zwischen den relativ kurzen

Zähnen. Eine andere Ähnlichkeit mit *S. vulgaris* besteht darin, dass die Blütenrispen nicht so überhängend wie bei *S. chinensis*, sondern mehr aufrecht sind.

Bei dieser Fliedersorte sind nun bei verschiedenen Gelegenheiten Zweige oder kleinere Partien von abweichendem Aussehen beobachtet worden. Schon ALEXANDER BRAUN (1873, 1874) erwähnt einige solche Fälle. Er hat an einem Exemplar in Berlin in zwei aufeinanderfolgenden Jahren Blüten gefunden, die nicht nur die lila Farbe von *S. chinensis* hatten, sondern auch der Form nach ganz mit dieser übereinstimmten. Sie kamen entweder in besonderen Rispen vor, wo alle Blüten vom *chinensis*-Typus waren, oder auch es konnte ein Blütenstand sektorial

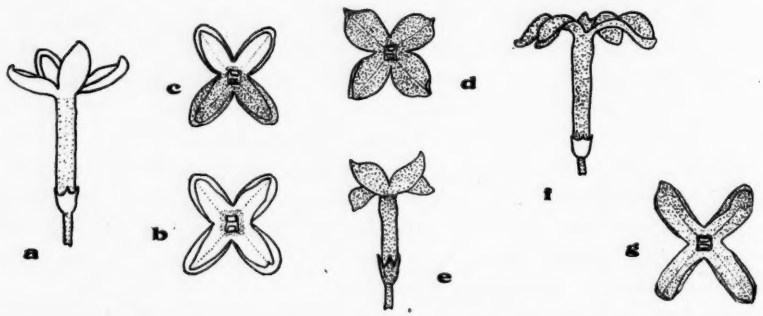

Fig. 1. Zwei Blüten der sog. *Syringa chinensis* var. *alba* (= *S. correlata*) im Botanischen Garten zu Lund (*a*—*b*), eine Blüte desselben Exemplares, die zur Hälfte lilafarbig ist (*c*), zwei *chinensis*-Blüten eines Rückschlagzweiges desselben Exemplares (*d*—*e*), zwei Blüten von *S. vulgaris* (*f*—*g*). — Schwach vergrössert (× 1⅔).

aufgeteilt sein, sodass ein Sektor vom gewöhnlichen Typus, ein anderer vom *chinensis*-Typus war und bestimmte Blüten sektorial in lila und blassfarbige Partien vom *chinensis*-, bzw. *alba*-Typus aufgeteilt waren. Ähnliche Abweichungen — vereinzelte Blüten oder auch Gesamtinfloreszenzen vom *chinensis*-Typus — werden u. a. von SCHÜBELER (1888) erwähnt, der sie mehrmals an in Norwegen gezogenen Exemplaren gefunden hat, und ferner von L. HENRY (1901), der in Frankreich in 7 verschiedenen Jahren an mehreren Exemplaren der Sorte von verschiedener Herkunft solche Abweichungen beobachtet hat.

An einem alten Exemplar der Sorte im Botanischen Garten zu Lund, das den gewöhnlichen Namen *S. chinensis* var. *alba* trägt, traten i. J. 1946 auch einige Abweichungen auf. An einem grossen Ast gab es in grosser Höhe, unweit des Gipfels, ein Zweiglein mit zwei Blüten-

rispen, die sowohl in der Farbe wie in der Gestalt der Blüten ganz mit *S. chinensis* übereinstimmten. Die Blüten waren nur etwas kleiner als bei *S. chinensis* gewöhnlich der Fall ist, aber auch die anderen Blüten auf dem Ast, der die Abweichungen trug, waren etwas kleiner als am übrigen Strauch; offenbar war der Ast schlecht ernährt oder aus anderer Ursache geschwächt. An einem anderen Ast kam in einer sonst typischen Blütenrispe eine vereinzelte Blüte vor, die halb lilafarbig, halb blass wie der Typus war. Die Grenze verlief über zwei entgegengesetzte Einschnitte des Saumes; es waren also zwei Abschnitte ganz lila, zwei von der Farbe der Varietät. Die Kronröhre ist ja auch bei dieser etwas gefärbt; man konnte aber deutlich sehen, dass genau die eine Längshälfte stärker gefärbt und scharf vom übrigen Teil abgegrenzt war; sie hatte übrigens einen hellen Mittelstreifen. Die diesem stärker gefärbten Kronenteil entsprechende Partie des Kelches war nur an der einen Seite in einem schmalen Band, ungefähr einem halben Kelchzahn entsprechend, vom *chinensis*-Typus; im übrigen stimmte der Kelch mit der Varietät überein. Der Gestalt nach wichen die lila Abschnitte dieser sektorial geteilten Blüte nicht merkbar von *S. chinensis* var. *alba* ab; sie waren etwas länger als die »weissen» Abschnitte, aber sonst hatten sie ungefähr dieselbe Form wie diese, mit ziemlich stark eingerollten Rändern. Dass sie nicht *chinensis*-Form hatten, ist vielleicht darauf zurückzuführen, dass die Aussenseite der Krone, wie der Kelch, möglicherweise zum grösseren Teil nicht vom *chinensis*-Typus war.

An einem Exemplar der Sorte in den städtischen Anlagen in Lund wurde i. J. 1946 ein grosser, kräftiger Ast von *S. chinensis* beobachtet; er zweigte indessen so tief unten am Stamm ab, dass man mit der Möglichkeit rechnen muss, dass er in der Unterlage, auf der der Strauch veredelt ist, seinen Ursprung hat; es braucht nicht unbedingt ein Rückschlag zu sein. Jedenfalls kommen Rückschläge zu *S. chinensis* nicht nur in seltenen Ausnahmefällen, sondern, wie besonders die Literaturangaben zeigen, ziemlich häufig bei der Sorte vor.

Als A. BRAUN seine Beobachtungen über die eigentümlichen Variationen bei *S. chinensis* var. *alba* — von ihm *S. correlata* genannt — vorlegte, führte er einige verschiedene Möglichkeiten zu ihrer Erklärung an, konnte aber keine ganz befriedigende Lösung des Rätsels finden — ganz natürlich in einer Zeit, wo die Natur der Periklinalchimären noch nicht klargelegt war. Heutzutage bietet es keine so grossen Schwierigkeiten, die Bildung der Form und die Ursachen ihrer vegetativen Aufspaltung festzustellen. Ohne Zweifel handelt es sich um eine Periklinal-

chimäre; dafür sprechen die hin und wieder erscheinenden Rückschläge zu *S. chinensis*, die oft bestimmte Sektoren in einer für Rückschläge der Periklinalchimären typischen Weise bilden, wie dies z. B. bei *Rhododendron Simsii* var. *Vervaeneanum* und *Crataegus media* var. *punicea* (HJELMQVIST, 1944) vorkommt. Die eine Komponente der Chimäre besteht, wie die Rückschläge zeigen, aus *Syringa chinensis*, mit typischer, lila Blütenfarbe. Die andere Komponente ist nicht auf Grund von Rückschlägen bekannt, lässt sich aber mit Hinblick auf das Aussehen der Blüten als eine Form von *S. vulgaris* mit weissen Blüten erwarten. Dass es sich um eine von *S. vulgaris* und *chinensis* gebildete Periklinalchimäre handelt, wird durch einige morphologische und anatomische Merkmale bestätigt, die auch über die Anteile der beiden Komponenten an der Chimäre Aufschluss geben.

Was zuerst den Kelch und die Blütenstiele betrifft, so bestehen zwischen *S. chinensis* und *vulgaris* bestimmte Unterschiede in der Beschaffenheit der Oberfläche, also in der Ausbildung der Epidermis. Bei *S. chinensis* ist der Kelch, der wie erwähnt eine rötliche Oberfläche hat, von sitzenden Drüsen punktiert, bei *S. vulgaris* ist er grün und auf der Oberfläche und am Rand mit gestielten Drüsenhaaren versehen; solche Haare sind auch auf den Blütenstielen vorhanden, fehlen aber bei *S. chinensis* auch hier. Die var. *alba* stimmt in diesen Merkmalen vollkommen mit *S. vulgaris* überein: der Kelch ist grün und Drüsenhaare gibt es sowohl hier wie auch auf den Blütenstielen.

In der Anatomie der Blätter bestehen einige Unterschiede, die zwar nicht gross sind, aber doch gewisse Schlüsse gestatten. Die Epidermis hat in bezug auf die meisten Merkmale bei allen drei Formen ähnliches Aussehen; Spaltöffnungen kommen z. B. bei allen reichlich auf der Unterseite, spärlich auf der Oberseite vor; Drüsen kommen ebenso bei allen beiderseits vor. In Flächenschnitten von der Unterseite des Blattes (Fig. 2 *a*—*c*) ist indessen zu sehen, dass die Seitenwände der Epidermiszellen bei *S. chinensis* beinahe völlig gerade sind; bei *S. vulgaris* dagegen sind sie einigermassen gekrümmt, oft unregelmässig gewellt. Die Varietät *alba* stimmt hierin, wie betreffs der Epidermis des Kelches und der Blütenstiele, mit *S. vulgaris* überein.

Hinsichtlich des Inneren der Blätter (Fig. 2 *d*—*f*) bestehen keine scharf markierten Unterschiede. Bei *S. vulgaris* sind jedoch die zwei Palisadenschichten gewöhnlich von fast derselben Grösse; die Zellen der unteren Schicht sind nur wenig kleiner als die der oberen, annähernd ebenso lang; bei *S. chinensis* sind die Zellen der unteren Palisadenschicht gewöhnlich erheblich kleiner als die der oberen, oft nur halb so lang.

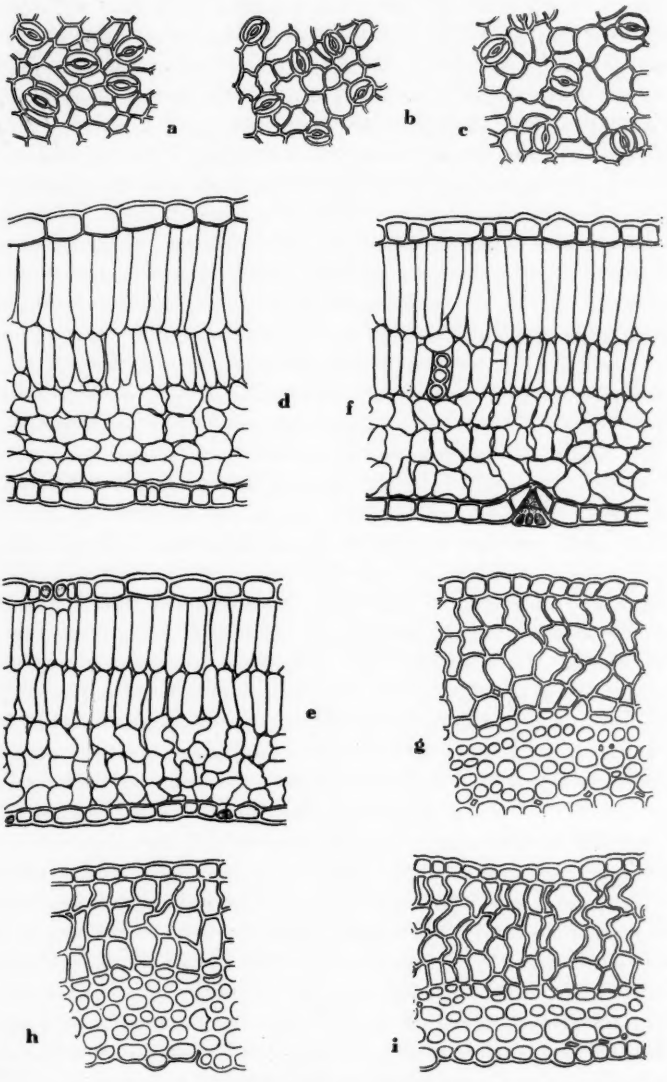

Fig. 2. Anatomische Unterschiede zwischen verschiedenen *Syringa*-Formen. — *a—c*: die Epidermis der Blattunterseite in Flächenansicht; *d—f*: Blattquerschnitte; *g—i*: Querschnitte durch den äusseren Teil einjähriger Zweige. — *a*, *f*, *g*: *S. chinensis*; *b*, *e*, *h*: *S. vulgaris*; *c*, *d*, *i*: die Chimäre. — Ca. 180fache Vergrösserung.

Dies ist auch bei var. *alba* der Fall, die also in diesem Merkmal mit *S. chinensis* übereinstimmt. Dieses Grössenverhältnis bildet zwar kein absolut unterscheidendes Merkmal — Variationen kommen auch an demselben Strauch vor —, aber wenn man nur Blätter unter denselben Umständen, Lichtverhältnissen usw. vergleicht und das vorherrschende Verhältnis im Auge behält, ist es doch möglich, einen Unterschied zu finden. Auch scheint das Schwammparenchym bei *S. chinensis* und var. *alba* etwas kompakter zu sein als bei *S. vulgaris*.

Auch in der Anatomie des Stammes kann man einige, wenn auch nicht grosse Unterschiede zwischen *Syringa vulgaris* und *S. chinensis* beobachten. In jüngeren Zweigen gibt es in der primären Rinde ein Kollenchymgewebe von ziemlich oberflächlicher Lage — nur durch ein ca. 3—4-schichtiges Parenchym von der Epidermis getrennt — und in diesem Kollenchym kann man vielleicht einige Unterschiede beobachten, wenn es auch bei jeder Form etwas variiert. Bei *S. vulgaris* (Fig. 2 *h*) sind die Zellen dieses Gewebes gewöhnlich ziemlich unregelmässig gelagert und mit ringsum ziemlich gleichmässig verdickten Wänden versehen. Das entsprechende Gewebe bei *S. chinensis* (Fig. 2 *g*) pflegt dagegen stark verdickte perikline Wände zu haben, während die antiklinen erheblich dünner sind, und die Zellen sind im allgemeinen regelmässig in konzentrischen Zellagen geordnet, die also durch starke konzentrische Membranleisten von einander getrennt werden. In derselben Weise verhält sich var. *alba* (Fig. 2 *i*). Im Mark des Stammes sind ferner die Zellwände bei *S. chinensis* und ebenso bei der var. *alba* fein und unregelmässig getüpfelt, während bei *S. vulgaris* von dieser Tüpfelung fast nichts zu sehen ist. Diese Unterschiede zwischen den beiden vorigen und der letzten Form kommen sowohl in einjährigen wie in älteren Zweigen und auch in den Infloreszenzachsen vor.

Betreffs verschiedener Organe stimmt also die Varietät *alba* im oberflächlichen Gewebe mit *S. vulgaris*, in inneren Geweben mit *S. chinensis* überein. Die Epidermis ist ohne Zweifel vom *vulgaris*-Typus; schon unmittelbar darunter scheint *chinensis*-Gewebe aufzutreten. Auf Grund der nicht allzu grossen Unterschiede zwischen *S. vulgaris* und *chinensis* kann vielleicht nicht behauptet werden, dass durch die Anatomie ganz bindend bewiesen ist, dass alle Zellagen unter der Epidermis von *S. chinensis* gebildet werden. Es geht auch nicht, durch Aufziehen von Samenpflanzen Auskunft über die Natur der Hypodermis zu gewinnen, denn die Form ist — wie im allgemeinen auch *S. chinensis* — steril und setzt keine Samen an. Es gibt indessen andere Tatsachen, die dafür sprechen, dass nur die Epidermis dem *vulgaris*-Typus angehört. Erstens ist ja

die Blattform ganz vom *chinensis*-Typus. Die Hauptmasse der Blätter geht ja aus der hypodermalen Zellage im Vegetationspunkt hervor; würde diese einer *vulgaris*-Form angehören, so würden die Blätter ein ganz anderes Aussehen bekommen; sie muss statt dessen zu *S. chinensis* gehören. Ferner ist von Untersuchungen des Vegetationspunktes bei *Syringa* durch A. SCHMIDT (1924) bekannt, dass hier in der Hypodermis nicht selten perikline Teilungen vorkommen. Aus diesem Grund ist SCHMIDT der Ansicht, dass eine diplochlamyde Chimäre bei *Syringa* kaum vorkommen, jedenfalls nicht Bestand haben kann. Es steht wenigstens fest, dass wenn eine solche entstehe, so würden Rückschläge zur äusseren Komponente sehr häufig vorkommen. Solche Rückschläge sind ja bei unsrer *Syringa*-Chimäre überhaupt nicht bekannt und sie kann darum nicht mehr als haplochlamyd sein.

Ich muss also zu dem Ergebnis kommen, dass *Syringa chinensis* var. *alba* eine Periklinalchimäre ist, bei der die unter der Epidermis liegenden Gewebe von der typischen *S. chinensis* mit lila Blüten gebildet werden, während die Epidermis von einer *S. vulgaris*-ähnlichen Form gebildet wird, die sich in keinem Rückschlag gezeigt hat, aber allem Anscheine nach die weissblütige *S. vulgaris* darstellt. Mit Rücksicht auf diese Zusammensetzung ist es ja nicht angemessen, die Form als Varietät zu *S. chinensis* zu rechnen, sondern gleichwie andere ähnlichen Chimären soll sie wohl einen eigenen Namen haben. Es scheint mir am richtigsten, den alten Namen A. BRAUNS *S. correlata* wiedereinzuführen.

Die Eigenschaften, die *Syringa correlata* kennzeichnen, lassen sich leicht mit Hilfe der Annahme erklären, dass sie eine haplochlamyde Chimäre von der erwähnten Zusammensetzung ist. Das *chinensis*-ähnliche Aussehen der Blätter hat darin seinen Grund, dass *S. chinensis* die hypodermalen Zellagen bildet, aus denen die Blätter wie oben erwähnt zum grösseren Teil hervorgehen. Es wäre vielleicht zu erwarten, dass die Epidermis auch einen Einfluss auf die Blattform zu Ähnlichkeit mit *S. vulgaris* haben sollte, wie bei *Crataegomespilus Asnieresi* und anderen haplochlamyden Chimären die Blattform auch von der äusseren Komponente bestimmt wird; dass dies nicht der Fall ist, wird jedoch dadurch erklärt, dass die Unterschiede in der Blattform zwischen *S. vulgaris* und *chinensis* sich auf Merkmale beziehen, die durch tiefere Zellagen bestimmt werden; sie beziehen sich ja meist auf die Gestaltung der Blattbasis. Dass die Blüte *vulgaris*-ähnlicher ist, findet darin seine natürliche Erklärung, dass Kelch und Blumenkrone, besonders ihre äusseren Partien, dünne Gebilde sind, die nur aus wenigen Zellagen bestehen, sodass die Epidermis dort eine relativ grössere Rolle spielt.

Es ist ja nicht unmöglich, dass die Randpartien wie bei gewissen anderen Blüten ganz von der Epidermis gebildet werden. Die Blütenfarbe ist wahrscheinlich durch miteinander konkurrierende Tendenzen in der Epidermis und den darunter liegenden Schichten zu erklären; diese haben Anlage für Anthocyanbildung in den Epidermiszellen; der Epidermis selbst fehlen vermutlich solche Anlagen gänzlich; dadurch erklärt sich, dass die dünnen Lappen des Saumes fast weiss werden, während die dickere Röhre etwas stärker gefärbt erscheint. Am meisten für die Farbe der Epidermiszellen entscheidend sind natürlich die eigenen Anlagen dieser Zellen; einigermassen können sie jedoch ganz sicher auch von den angrenzenden Zellagen beeinflusst werden, wahrscheinlich durch Zufuhr von Stoffen, die für die Anthocyanbildung nötig sind (vergl. *Rhododendron Simsii* var. *Vervaeneanum* u. a.; HJELMQVIST, 1944).

Dass Rückschläge zu *S. chinensis* ziemlich häufig sind, während Rückschläge zur äusseren Komponente sehr selten sind, wenn sie überhaupt vorkommen, steht auch mit der haplochlamyden Natur der Chimäre im Einklang; bei haplochlamyden Chimären pflegen Rückschläge zur inneren Komponente erheblich häufiger aufzutreten als solche zur äusseren.

Über die Entstehung von *S. correlata* ist nichts Sicheres bekannt. Die ältesten Literaturangaben, die diese Form betreffen, dürften die Berichte A. BRAUNS (1873, 1874) über ein altes Exemplar von *S. correlata* im Berliner Botanischen Garten sein, das seinerzeit von einem Gärtner in Halle erhalten wurde. Nach Angabe war es auf *S. chinensis* gepfropft. Es erscheint möglich, dass die Form bei dieser Pfropfung entstanden ist, sodass die eingepfropfte Sorte eine *vulgaris*-Form, wahrscheinlich mit weissen Blüten, gewesen ist, und dass von der Pfropfstelle eine Chimärenbildung ausgewachsen ist. Es ist ja auch möglich, dass die Form früher entstanden ist; jedenfalls dürfte die Entstehung in ähnlicher Weise stattgefunden haben.

Nach Angaben von E. OTTO (1859) und SCHÜBELER (1888) ist an *Syringa chinensis* einmal ein Ast beobachtet worden, der mehrere Blütenrispen trug, die augenscheinlich völlig mit *S. persica* übereinstimmten. In einem anderen Fall ist ein »Sport» von einer anderen *chinensis*-Sorte, var. *Saugeana*, aufgetreten (HENRY, 1901). Ist denn auch *S. chinensis* eine Chimäre? Wegen der grossen Seltenheit solcher Veränderungen ist dies nicht anzunehmen. Übrigens ist hervorzuheben, dass nach späteren Untersuchungen (TISCHLER, 1930) die als *S. persica* angebaute Form tatsächlich gleichwie *S. chinensis* eine Hybride zwischen

der Wildform von *S. persica* und *S. vulgaris* darstellt; die erwähnten Abänderungen stellen also nur Übergänge von einer Form zu einer anderen innerhalb derselben Hybridenserie dar; wie sie erklärt werden sollen, ist wohl den Zytologen zur Entscheidung zu unterbreiten.

Bei einer Farbenvarietät von *Syringa chinensis*, var. *metensis*, scheinen jedoch nach den Angaben der Literatur Rückschläge zur typischen Blütenfarbe ziemlich häufig vorzukommen (Mc KELVEY, 1928, S. 421; nach L. HENRY). Vermutlich handelt es sich hier um eine Chimäre; selbst habe ich diese Form nicht untersucht. Ihre Entstehung ist genau bekannt; sie entstand als ein »Sport» auf einem Exemplar von *S. chinensis* in Metz um das Jahr 1860 (Mc KELVEY, a. a. O.). Wenn diese Form — wie zu vermuten ist — eine Periklinalchimäre darstellt, dann ist sie also eine Mutationschimäre, in der die beiden Komponenten sich nur in der Blütenfarbe von einander unterscheiden; sie ist also von einem anderen und gewöhnlicheren Typus als *S. correlata*, die zu der seltenen Gruppe der echten »Pfropfbastarde» gehört.

SUMMARY.

A periclinal chimaera in the genus Syringa. — An old garden-form of *Syringa*, generally called *S. chinensis* var. *alba*, is often reported to give inflorescences, flowers or flower-sections of the typical *S. chinensis* (BRAUN, SCHÜBELER, HENRY), and the author has also seen such reversions in the Botanical Garden of Lund. In one case only half a flower had the colour of *S. chinensis* (Fig. 1 *c*). The form has leaves that are of quite the same shape as in *S. chinensis*, whereas the flowers (Fig. 1 *a—b*) are more reminiscent of *S. vulgaris* (Fig. 1 *f—g*) in the cucullate flower-lobes and the round incisions of the calyx. Its flowers are of a faint lilac colour, especially pale in the lobes. The reversions (Fig. 1 *d—e*) are typical *S. chinensis* in shape as well as in colour. This makes it probable that the plant is a chimaera of this species and a *vulgaris*-like form. Confirmation of this is afforded by morphological and anatomical facts. The epidermis of the var. *alba* agrees with *S. vulgaris*. In the calyx and the pedicels it is green and glandular hairy as in this species, while *S. chinensis* has a reddish calyx with glandular dots there as well as on the pedicels. The lower epidermis of the leaves (Fig. 2 *a—c*) has — seen from the surface — curved walls in var. *alba* as in *vulgaris*, in *S. chinensis* almost quite straight ones. Under the epidermis the tissues of var. *alba* agree with *S. chinensis*; the differences

between these two forms and *S. vulgaris* are, however, not great. In *S. chinensis* and var. *alba* the proportion between the two palisade layers in the leaves is generally different from that in *S. vulgaris* (Fig. 2 *d—f*), and the collenchyma of the bark has generally more regular layers and thicker periclinal walls in relation to the anticlinal ones (Fig. 2 *g—i*). These facts, together with the meristematic conditions of *Syringa*, elucidated by SCHMIDT, speak in favour of the var. *alba* being a haplochlamydous chimaera. The inner component is *Syringa chinensis*, the outer probably a white *S. vulgaris*. Under these circumstances it should have a name of its own, most correctly the old name of A. BRAUN, *S. correlata*. It has probably arisen through grafting of *S. vulgaris* on *S. chinensis*.

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COMBINATION EXPERIMENTS WITH MUTANT STRAINS OF OPHIOSTOMA MULTIANNULATUM

BY *NILS FRIES* AND *ULLA TROLLE*
INSTITUTE OF PHYSIOLOGICAL BOTANY OF THE UNIVERSITY OF UPPSALA, SWEDEN

THE fungus that was the object of the following study was isolated in 1924 by HEDGCOCK from pine lumber, and described in 1935 by DAVIDSON and HEDGCOCK (DAVIDSON, 1935) under the name of *Ceratostomella multiannulata*, a name, however, which according to NANNFELDT (MELIN and NANNFELDT, 1934) should be exchanged for *Ophiostoma multiannulatum*. The species in question is obviously attached to the earlier known *O. pluriannulatum* (HEDGC.) H. and P. SYD., and like this (GREGOR, 1932) it is heterothallic (ANDRUS, 1936). As is shown by ROBBINS and MA (1942) as well as by FRIES (1943), *O. multiannulatum* is capable of assimilating a synthetic nutrient solution if — besides sugar and the usual inorganic salts — it contains vitamins thiamin (vitamin B_1) and pyridoxin (vitamin B_6). By x-ray irradiation, however, it is possible to produce mutations requiring a further addition of some vitamin, a purin, a pyrimidine, etc., to the substratum (FRIES, 1945, 1946 b).

The interesting observations of BEADLE and COONRADT (1944) as to the formation of heterocaryotic mycelia in *Neurospora crassa* led us to investigate whether heterocaryosis of the same type could appear in *Ophiostoma* too. As components of the combination experiments some mutants (or descendants of mutants) were chosen, characterized by their inability to synthesize uracil (Nos. 460, 1178 and 1208), hypoxanthine (Nos. 513, 636, 870), adenine (Nos. 1174 and 1202), or guanine (No. 848), all of them obviously without a tendency towards adaptation.

The isolation of hyphal tips was performed in the following way. Dry sterilized cover-glasses were immersed into a hot, sterile agar-solution consisting of 1,5 % washed agar, 0,2 % glucose, 0,5 % NH_4-tartrate, 0,1 % KH_2PO_4, 0,05 % $MgSO_4 \cdot 7H_2O$, 0,01 % NaCl, 0,01 % $CaCl_2$, 40 γ/lit. of vitamin B_1, 40 γ/lit. of vitamin B_6, and traces of Fe, Zn and Mn. Immediately after that, every cover-glass was put into a sterile petri dish, where the nutrient solution solidified into a thin film on the

surface of the cover-glass. A piece of the mycelium which was to be investigated was inoculated on this film. By using a thin agar film and a percentage of glucose ten times lower than that otherwise used, the mycelium growing out on the cover-glass became so loose that, by the aid of a platinum needle, individual tips of hyphae could be isolated directly under the microscope.

In isolating a hyphal tip a square piece was cut out of the agar coat in which the tip was growing, and this piece was transferred to a petri dish containing transparent malt-agar (impurities were removed by centrifugation). The growth of the hypha on the agar plate was followed by a microscopic examination every sixth hour, by which it could be controlled that no other tip of hypha, or any conidium, had through an oversight been carried along in the isolation. When the tip had ramified and grown out to a suitable length in the surrounding malt-agar, it was isolated once more with a platinum needle in the same way as before. The piece with the hyphal tip thus isolated twice was finally transferred to a culture tube containing malt-agar.

The isolation of conidia was carried out in the usual manner (FRIES, 1946 a, p. 128).

In the first experiment two (—)-mycelia, 513: 14 (hypoxanthine-heterotrophic) and 848: 20 (guanine-het.), were combined on malt-agar. A mixed mycelium was obtained, and when an inoculum of this was transferred to a synthetic nutrient solution, it grew there quite well. Eight cultures obtained from isolated unramified hyphal tips behaved in the same manner. Since it could be the question of heterocaryotic mycelia, also ten conidia were isolated and allowed to grow out into mycelia. These too, however, turned out to react as quite normal mycelia of the »wild type». Either the conidia were heterocaryotic too, or a reduction division had occurred resulting in a segregation of physiologically normal ascospores — prototrophic, according to the terminology of RYAN and LEDERBERG (1946). The former alternative being true, the conidia must contain several nuclei, which was not very probable, the latter alternative presupposed an »illegitimate» copulation since the components of the mycelium both represented the same incompatibility group.

In combining two (+)-mycelia, 848: 5 (guanine-heterotrophic) and 460 (uracil-het.), a result was won which wholly agreed with that just described.

If the conidia and the mycelia developed from these (in the experiment first reported) were heterocaryotic, some of the ascospores

produced by mating such a mycelium with a prototrophic strain must give rise to hypoxanthine-heterotrophic mycelia, some other ascospores must produce guanine-heterotrophic mycelia, and the remaining spores, prototrophic mycelia. One of the above-mentioned 1-conidium-mycelia, obtained from the combination 513: 14 + 848: 20, produced perithecia with the (prototrophic) test-mycelium No. 1 (+). Seventy-nine 1-ascosporous mycelia were isolated, and their demand for purins was tested. It appeared that all were prototrophic. As ascospores have one nucleus each, heterocaryosis is excluded. Consequently both parental mycelia — thus the 1-conidium-mycelium too — must have been prototrophic and not heterocaryotic.

An illegitimate copulation must accordingly have occurred between the two mutant (—)-mycelia in the combination culture mentioned above. To get an idea of the frequency of such illegitimate copulations, 37 combinations were made between different uracil-, hypoxanthine-, adenine- and guanine-heterotrophic mutants belonging to the same incompatibility group. In some cultures, formations were observed which could be presumed to be rudiments of fruit-bodies. Completely developed fruit-bodies did not appear in any single case, and have in fact never been observed in our laboratory in monosporic cultures, or in cultures of the (+) × (+)- or (—) × (—)-type. In this respect *O. multiannulatum* wholly corresponds to *Neurospora*.

When the combination cultures in question were 50 days old, two big pieces of the mycelial mat together with the agar layer immediately beneath were removed, and one was transferred to a flask containing a sterile, synthetic nutrient solution (without any addition of nucleotide components), the other was fixed in pikroformol, according to BOUIN-MAIRE. In 23 of the flasks inoculated in this manner the inoculum remained rather unaltered; only by degrees did solitary hyphae slowly develop; in 14 flasks, on the other hand, an intensive development of mycelium immediately set in, so that the whole volume of the solution was interwoven with hyphae even in a few days (Table 1). In the latter cases the combination cultures had given rise to prototrophic mycelia. In some of these cases it was controlled that it was not the question of heterocaryotic mycelia.

The fixed pieces of mycelium were microtomized, stained with iron haematoxyline and lichtgrün, and examined as to the occurrence of rudiments of perithecia. Such rudiments turned out to be present in all the cultures giving rise to prototrophic mycelia. Thus, there hardly seems to be any doubt about the prototrophic mycelia having arisen

TABLE 1. *The segregation of prototrophic mycelium in illegitimate combinations between different mutant strains of Ophiostoma multi-annulatum, and the growth rate of the combinations on agar with minimum nutrient solution.*

a = adenine-heterotrophic; g = guanine-heterotrophic; h = hypoxanthine-heterotrophic; u = uracil-heterotrophic.

In the second column + means that prototrophic mycelium was developed, — means that no development of prototrophic mycelium was observed. In the last column . means that no determination of the growth rate was made.

Strains	Development of prototrophic mycelium	Growth rate of the combination in mms. per day
513 (+, h) + 848: 5 (+, g)	—	0
513 (+, h) + 848: 15 (+, g)	+	0
636 (+, h) + 848: 5 (+, g)	—	0,8
870 (+, h) + 848: 5 (+, g)	—	0
870 (+, h) + 848: 15 (+, g)	+	0
513: 1 (—, h) + 848 (—, g)	—	0
513: 14 (—, h) + 848 (—, g)	—	0
513: 1 (—, h) + 848: 20 (—, g)	—	0
513: 14 (—, h) + 848: 20 (—, g)	+	2,1
1174 (+, a) + 848: 5 (+, g)	—	.
1174 (+, a) + 848: 15 (+, g)	—	.
1202 (+, a) + 848: 5 (+, g)	—	0
1202 (+, a) + 848: 15 (+, g)	—	0
513 (+, h) + 460 (+, u)	—	0,9
513 (+, h) + 460: 9 (+, u)	+	0
513 (+, h) + 1178 (+, u)	+	0
513 (+, h) + 1208 (+, u)	—	0
870 (+, h) + 460: 9 (+, u)	+	0,5
870 (+, h) + 1178 (+, u)	+	0
870 (+, h) + 1208 (+, u)	—	0,4
513: 1 (—, h) + 460: 4 (—, u)	—	0
513: 14 (—, h) + 460: 4 (—, u)	—	0
1174 (+, a) + 460: 9 (+, u)	—	.
1174 (+, a) + 1178 (+, u)	—	.
1174 (+, a) + 1208 (+, u)	—	.
1202 (+, a) + 460: 9 (+, u)	—	0

Strains	Development of prototrophic mycelium	Growth rate of the combination in mms. per day
1202 (+, a) + 1178 (+, u)	—	0
1202 (+, a) + 1208 (+, u)	—	0
848: 5 (+, g) + 460 (+, u)	+	.
848: 5 (+, g) + 460: 9 (+, u)	+	0,7
848: 5 (+, g) + 1178 (+, u)	—	0,7
848: 5 (+, g) + 1208 (+, u)	+	0,3
848: 15 (+, g) + 460: 9 (+, u)	+	0,5
848: 15 (+, g) + 1178 (+, u)	+	0,5
848: 15 (+, g) + 1208 (+, u)	+	0,2
848 (—, g) + 460: 4 (—, u)	—	0,6
848: 20 (—, g) + 460: 4 (—, u)	+	1,0
1 (+, prototrophic)	(+)	4,1
2 (—, prototrophic)	(+)	4,7

from ascospores formed after reduction division. Such illegitimate copulations and reduction divisions are, however, obviously rare. No clear reduction division could be observed in any of the slides, nor did a segregation of normal mycelia take place in all the cultures where rudiments of perithecia appeared.

Thus, the existence of illegitimate copulations in *O. multiannulatum* would undoubtedly have been very difficult to establish, unless the copulants combined here had been of such a sort that a certain product of segregation — the prototrophic mycelium — could easily be identified by its physiological properties.

In the experiments mentioned the illegitimate copulations rendered it however impossible to decide whether heterocaryosis occurs in *O. multiannulatum*. To prevent these copulations, if possible, experiments were made to combine different types of mutants on a synthetic agar medium of the composition stated on a foregoing page, but with 2 % glucose instead of 0,2 %. The growth of the mutants on this substratum was extremely poor and was caused by occurrence of inconsiderable traces of the respective nucleotide constituents in the inocula and in the substratum. Rudiments of fruit-bodies did not occur in any case. In the 31 combination cultures arranged with different mutants (Table 1) a rather vigorous formation of mycelium set in after

two weeks, at the earliest, in 13 of the cultures. The growth rate was, however, very low, and considerably inferior to the prototrophic mycelia on this substratum.

One of these combination cultures, 636 (+, hypoxanthine-heterotrophic) × 848: 5 (+, guanine-het.), was more closely examined. All of 9 isolated hyphal tips as well as all the conidia developed into hypoxanthine-heterotrophic mycelia. Consequently, no reduction division had taken place in this case, nor had a heterocaryotic mycelium arisen; but the growth was probably conditioned by an extracellular interchange of hypoxanthine and guanine in the same way as has earlier been described in so-called artificial symbioses (KÖGL and FRIES, 1937; SCHOPFER, 1938; »extracellular symbiosis», according to BEADLE and COONRADT, 1944). That no guanine-heterotrophic conidia and tips of hyphae, but merely hypoxanthine-heterotrophic ones were obtained, probably depends on the fact that the hypoxanthine-heterotrophic component is the more vital and rapidly growing one in the combination. Presumably the guanine-heterotrophic partner limits the growth of the system.

In the combination between a hypoxanthine- and a uracil-heterotrophic mutant, viz. 513 (+) and 460 (+), all the four hyphal tips and nine conidia isolated were also hypoxanthine-heterotrophic. This component, then, is the most rapidly growing one even in this combination.

The possibility can of course not be excluded that also heterocaryotic hyphae are present in these combination cultures. It is obvious, however, that heterocaryotic mycelia having a growth rate equivalent to that of prototrophic mycelia cannot be formed in *O. multiannulatum* — unlike *Neurospora* — at least not under the conditions tested here. This may perhaps be due to the fact that the species of *Ophiostoma* — to judge from available particulars and figures in literature (MITTMANN, 1932; ANDRUS, 1936) — possess uninucleate cells, whereas *Neurospora*, like perhaps most other Ascomycetes, is a coenocyte with numerous nuclei in each cell.

The capacity of forming heterocaryotic mycelia characteristic of *Neurospora* has, as is known, proved to imply a very convenient means of deciding whether two gene mutations concern one and the same gene. This possibility seems to be excluded in *O. multiannulatum*. So in the first place one is here restricted to using the ordinary method with matings between mutants (or descendants of mutants) belonging to different incompatibility groups and to an examination of the properties of the progeny — fairly easily done, it is true, when it mainly

comes to indicating the absence or presence of physiologically normal, prototrophic products of segregation. From a certain mutant mycelium it may, however, often be difficult and time-consuming to produce — by crossings with normal test-mycelia — descendants of the opposite mating-type necessary for such combination experiments. In so far as illegitimate copulations between mutants within the same incompatibility group prove the existence of non-allelism by a segregation of prototrophic mycelium this roundabout way in solving such problems can be avoided.

Summary. — In 14 of 37 illegitimate combinations of different physiological (uracil-, hypoxanthine-, adenine- and guanine-heterotrophic) mutants of *Ophiostoma multiannulatum* on malt-agar a prototrophic (= »wild type») mycelium developed. It is supposed that this must have arisen through a segregation in rudiments of perithecia. Heterocaryotic hyphae could not be found in these combinations, nor in such combination cultures on agar containing a synthetic nutrient solution. In 13 of these 31 cases last mentioned, however, there was some growth probably due to the formation of extracellular symbioses.

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CONSTITUTION AND C-MITOTIC ACTIVITY OF ISO-COLCHICINE

BY *ERNST STEINEGGER* AND *ALBERT LEVAN*
CYTO-GENETIC LABORATORY, SVALÖF

I. INTRODUCTION.

COLCHICINE was isolated from *Colchicum autumnale* for the first time by ZEISEL. Through the work of this author, continued during a long period (ZEISEL, 1883, 1886; JOHANNY and ZEISEL, 1888; ZEISEL and v. STOCKERT, 1913), the empirical formula of colchicine was determined as $C_{22}H_{25}O_6N$. The function of the nitrogen atom as well as of five of the oxygen atoms was understood. The work of WINDAUS (e. g., 1924) and collaborators led to the constitution of formula (I) (see Scheme 1), which formula, however, does not account for all the properties of colchicine.

That colchicine has a remarkable action on mitosis was early known. Its cytological effect was studied in animal material by DUSTIN (e. g., 1934), in plant material by GAVAUDAN and GAVAUDAN (1937) and by DUSTIN, HAVAS and LITS (1937), and later on by a great many workers. The main effect of colchicine on mitosis is a more or less complete destruction of the spindle apparatus. The chromosomes divide as normally, but their daughter halves are not distributed to the poles. This deviation from the normal course of mitosis gives a very characteristic morphological picture, which in its main features is identical in all animals and plants studied. It was called c-mitosis by LEVAN (1938), which rather neutral term seems preferable to the more or less descriptive or hypothesizing terms, e. g., caryoclastic mitosis, stathmokinesis, polyploidogenic action, mitosis inhibition, mitosis poisoning, each of which may cover only certain sides of the reaction.

During later years a whole literature has grown up around the colchicine problem, especially since the polyploidogenic action of colchicine was detected by BLAKESLEE (1937). Most of these papers deal with cyto-genetic aspects, as the appearance and behaviour of the new artificially produced polyploids. The morphology of the cytological effect on the living cell has also received due attention, most workers being in agreement as to the main features of the c-mitosis.

Among the lines of research the outcome of which cannot yet be regarded as settled, reference may be made to the important question as to what part of the colchicine molecule is responsible for its activity (BRUES and COHEN, 1936; COOK and ENGEL, 1940; LETTRÉ, ALBRECHT and FERNHOLZ, 1941; LETTRÉ and FERNHOLZ, 1943). Other recent investigations have centred around the striking lack of specificity of the c-mitotic reactions. It has been found that many substances of quite different chemical character from colchicine nevertheless show c-mitotic activity. Thus, acenaphthene was found to be active (KOSTOFF, 1938) and likewise apiol (GAVAUDAN and GAVAUDAN, 1939). LEVAN and ÖSTERGREN (1943) and ÖSTERGREN and LEVAN (1943) tested series of related substances, and by means of a quantitative method it was possible to decide that as a rule some concentrations of most substances gave the reaction. This was true of all halogen and methyl derivatives tested of benzene, naphthalene, cyclohexane and thiophene. Activity was present especially in substances known for their narcotic properties (ÖSTERGREN, 1944). The apparent contradiction between, on one hand, specifically acting chemical groups and, on the other hand, the striking lack of specificity of the c-mitotic reactions may be overbridged, as will be discussed further in a later chapter.

The present writers intend to deal in a few papers with some problems connected with the c-mitosis, problems often lying on the borderline between biochemistry and cytology. This first communication will give some data concerning the action on *Allium* roots of an isomeric form of colchicine. The method employed is the same as earlier adopted at this laboratory, i. e. a determination of the effect of different concentrations of the substance on growing root-tips of *Allium Cepa* bulbs. Fixation of the root tips has been made at different intervals after the beginning of the treatment.

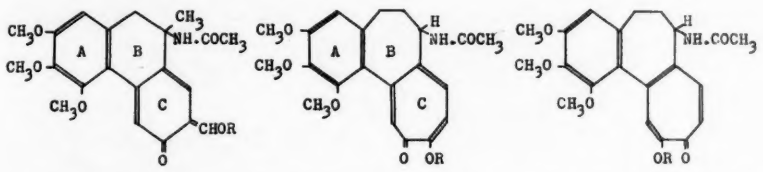
Our thanks are due to Dr. M. SORKIN, Basle, for presenting us with a sample of *iso*-colchicine. The stay of E. STEINEGGER at Svalöf has been made possible by a fellowship from the Swiss »Stiftung für biologisch-medizinische Stipendien».

II. THE CHEMICAL CONSTITUTION OF COLCHICINE AND ISO-COLCHICINE.

It has been shown that the constitution of colchicine as given by WINDAUS is unsatisfactory. BARTON, COOK and LOUDON (1945) claim that the ring *B* of colchicine must be seven-membered. DEWAR (1945 a,

b, c) supposes that the ring *C* is also seven-membered, just as is the case with the stipitatic acid isolated from *Penicillium stipitatum*. Through combination of the two seven-rings *B* and *C* DEWAR (1945 b) proposed the constitution (III) as being the most probable one for colchicine.

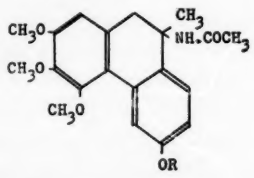
The methyl group of ring *C* is easily split off, colchiceine thereby originating constitution (II) according to WINDAUS, (IV) or (VI) according to DEWAR. While the constitution (II) of WINDAUS leaves open only one possibility for the formula of colchiceine (the possibility of cis-trans-



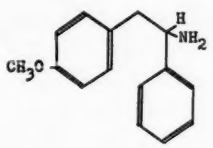
I R = CH_3 Colchicine
II R = H Colchiceine (WINDAUS)

III R = CH_3 Colchicine
IV R = H Colchiceine (DEWAR)

V R = CH_3 iso-Colchicine
VI R = H Colchiceine (DEWAR)



VII R = H N-Acetylcolchinol
VIII R = CH_3 N-Acetylcolchinol methyl ether



IX α-Phenyl-β-(p-methoxy-phenyl)-ethylamine

Scheme 1. The constitution of colchicine and related substances.

isomery is not taken into account), the constitution given by DEWAR allows of two resonating structures. This is perfectly analogous to the two resonating constitutions known for stipitatic acid (DEWAR, 1945 a).

If colchiceine is methylated, two isomeric methylated compounds would be expected, as indeed they have been obtained from stipitatic acid. Their structures would correspond to formulae (III) and (V). MEYER and REICHSTEIN (1944), after methylating colchiceine with diazomethane, obtained crystalline colchicine to a yield of two per cent with an optical rotation of $[\alpha]_D^{19} = -122{,}4° \pm 2°$, while the bulk of the reaction product remained amorphous and showed an optical rotation of

$[\alpha]_D^{24} = -223{,}5° \pm 2°$. The latter substance showed the same analytical composition as colchicine.

Recently SORKIN (1946) succeeded in separating two substances from the mixture originating after the reaction of diazomethane on colchiceine. Besides normal colchicine one isomere of colchicine, called *iso*-colchicine, was also obtained in crystalline form for the first time. *Iso*-colchicine is described by SORKIN as a colourless powder, crystallizing into orthogonal or hexagonal plates. Melting point: 225—226°, optical rotation: $[\alpha]_D^{12} = -306{,}7° \pm 3°$. In similarity to colchicine it dissolves in aqueous mineral acids with yellow colour. On heating together with mineral acids it again gives colchiceine.

III. THE C-MITOTIC ACTION OF ISO-COLCHICINE.

The action on *Allium* roots of a dilution series of *iso*-colchicine, including 7000, 1000, 100 and 10×10^{-6} mol/l, immediately revealed one striking difference from colchicine. In normal colchicine concentrations of 100 to 150×10^{-6} mol/l already give c-mitosis, in *iso*-colchicine none of the above concentrations gave complete c-mitosis. In the strongest, 7000×10^{-6} mol/l, there were tendencies to c-mitosis. Fig. 1 *b—d* represents three stages of increasing c-mitotic tendencies after four hours' treatment with 1000×10^{-6} mol/l (*b*) and with 7000×10^{-6} mol/l (*c—d*). Compared with the normal mitosis of Fig. 1 *a*, taken from a 24 hours' treatment with 10×10^{-6} mol/l, it will be noticed that the chromosome contraction is more pronounced in 7000×10^{-6} mol. Even the spindle begins to show irregularities. In *b* it is rather regular, in *c* the poles are flattened out, showing traces of multipolarity. The chromosomes are still gathered in an equatorial plate. In *c* this plate is quite irregular. Since the plasm in this cell showed a heavy staining, the spindle fibres were well visible. Several individual poles were present, and those small bundles of spindle fibres which are in contact with the centromeric regions of the chromosomes were oriented in different directions. Complete destruction of the spindle did not occur in this concentration.

On fixing after 24 hours' treatment it turned out that all concentrations, even the strongest one, showed exclusively normal mitoses. The c-mitotic effect shown by 7000×10^{-6} mol/l shortly after the beginning of the treatment thus depended on a shock action, early disappearing. No poison effect was noticed in these concentrations. The

roots were still turgescent after seven days, even if no further growth had occurred.

After the above results were known two higher concentrations were

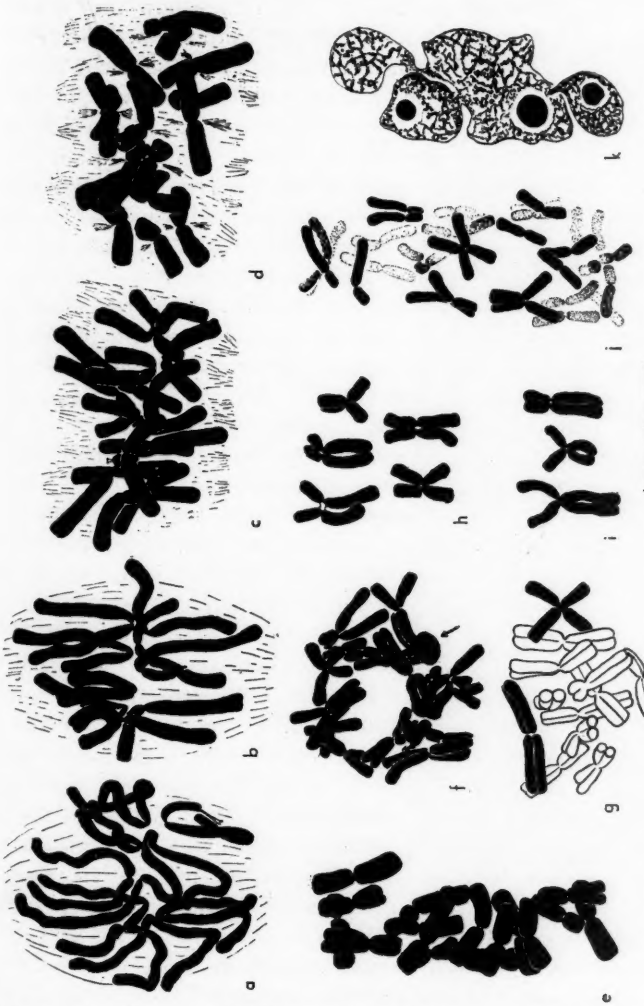


Fig. 1. *Allium Cepa* mitoses in the root-tips. — *a*: normal metaphase; *b—d*: increasing c-mitotic effect on the chromosomes; *e—g*: full c-metaphase; *h—i*: c-pairs from two cells, separately drawn; *j*: c-anaphase; *k*: lobated resting nucleus resulting from c-mitosis; *a*: 10, *b*: 1000, *c—d*: 7000, *e—k*: 14000 $\times$ 10^{-6} mol *iso*-colchicine; *b—e*, *g*, *k*: 4 hours', *a*, *f*, *h—j*: 24 hours' treatment. — $\times$ 2000.

tested, viz. 25000 and 14000 $\times$ 10^{-6} mol/l, i. e. 1 and 0,56 %. The higher of these, which corresponds to a semi-saturated solution, gave a distinct poison effect after 4 hours, and only few mitoses were present. These

were to a large extent c-mitoses. A decided stickiness was seen, the mitoses belonging to the compact, agglutinated type. After 24 hours no divisions were found in this concentration, after two days a few mitoses were again found, peculiarly enough they now had normal spindles and did not show especially strong contraction. After four days most of the meristem did not take the stain and the nuclei had no visible structures. The cells were probably not dead, however, the roots still remaining turgescent after 11 days. After 22 days the roots began softening, and no growth occurred.

The cytological study of the concentration 14000×10^{-6} was started after two hours' treatment. The mitoses were then normal but showed clear c-mitotic tendencies. After four hours most of the mitoses had turned into typical c-mitoses. Fig. 1 *e* is an instance of such a mitosis, the chromosomes of which are strongly contracted; the chromosomes show no equatorial arrangement and no traces of any spindle can be detected. The centromeres are probably still undivided, although each centromere is parted into two darker grains lying in line with the two chromatids. Between these stained parts the centromeres are lighter. It may be that the centromeres are already divided, the chromosomes, however, being held together by their stickiness.

A period of 24 hours in 14000×10^{-6} mol/l induces complete c-mitosis; normal spindles are totally absent in the slide. Different stages of the c-mitosis resulting from this treatment are pictured in Fig. 1 *f* and *h—j*. The c-mitosis here must be judged as complete and typical. It is characterized, however, by a higher degree of stickiness than is present in c-mitosis of, for instance, normal colchicine. This stickiness brings about an agglutination of the c-pairs of the c-metaphase. Sometimes two or more c-pairs stick together in one end at a drop of chromatin, which seems to have developed from the chromosome arms involved (see Fig. 1 *f*, at the arrow). A certain irregularity perceived in the development of the c-pairs is probably due to this stickiness. Thus, in the same cell there often occurred c-pairs with their chromatids quite parallel, sticking together along their whole length, besides typical x-shaped c-pairs. Sometimes the daughter chromatids of one c-pair may stick together in one half and be separated in the other half (Fig. 1 *h*, *i*). Such irregularities were still present after four days, as shown by Fig. 1 *g*. This shows one cell with one x-shaped c-pair, while all the other c-pairs have their chromatids sticking together parallelly. This difference in the appearance of the c-pairs may be of the same nature as that found by WITKUS and BERGER (1944). In their experiments

colchicine gave the ordinary x-shaped c-pairs, while veratrine gave c-pairs of the more sticky type.

In Fig. 1 *j* a c-anaphase is represented. At this stage, too, the course is somewhat more irregular than in ordinary c-mitosis. Most c-pairs of the picture are already separated into »ski pairs», but one or a couple are of x-shape and evidently hang together at their centromeres. Probably as a result of the stickiness, the division of the centromeres does not cause a simultaneous partition of the chromosomes. Or else the heavy treatment has upset the synchronization of the division of the centromeres. In normal c-mitoses the c-anaphase always starts absolutely at the same time in all c-pairs, while in c-mitosis induced by *iso*-colchicine the timing of the process seems to be less exact.

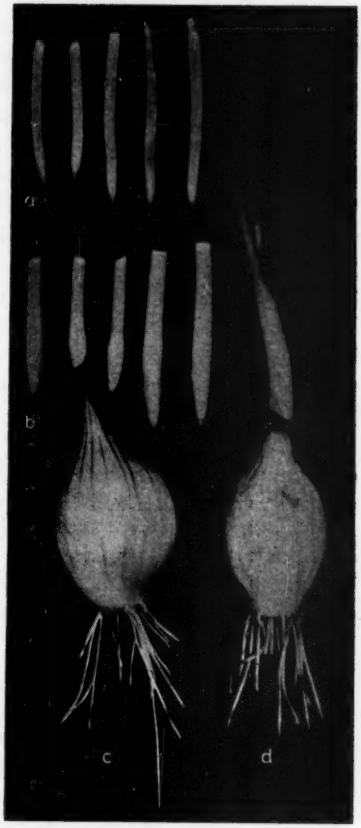


Fig. 2. *Allium Cepa.* — *a*, *c*: untreated, *b*, *d*: treated with 14000×10^{-6} mol *iso*-colchicine; *a*, *b*: enlarged root-tips from *c* and *d* respectively.

Fig. 1 *k* shows one lobated nucleus formed from a c-telophase in which the c-pairs must have been widely scattered. This type as well as multinuclear cells commonly occurred after a treatment of 14000×10^{-6} mol/l for four days. A slight poison effect was met with in this treatment, but the roots maintained their turgescence all the time. Most mitoses were of c-type at this time. The macroscopical colchicine reaction, the swelling-up of the growth region of the roots, the so-called c-tumour reaction, was carefully looked for all through our experiments. The reaction was found only in one concentration, viz. 14000×10^{-6} mol/l, in which it began to appear after three days. Fig. 2 shows the appearance of the c-tumours after five days. Undoubted c-tumours are seen clearly in the

enlarged roots of Fig. 2 *b*, especially when compared with the untreated roots of Fig. 2 *a*. The reaction is, however, less pronounced than after treatment with ordinary colchicine; the tumours are smaller and their shape is more elongated. It must be considered of some interest that the two reactions, c-mitosis and c-tumour, accompany each other in *iso*-colchicine just as in colchicine, although in the case of *iso*-colchicine in 100 times higher concentrations.

Here follows a survey of the observed occurrence of c-mitosis in *iso*-colchicine (+ = c-mitosis, ± = mixed c- and normal mitosis, — = normal mitosis, 0 = not studied):

Concentration in 10^{-6} mol/l	2 hours	4 hours	24 hours	2 days	4 days
25000	0	$\overset{+}{(-)}$	?	(—)	?
14000	$\overset{(+)}{-}$	±	+	0	$\overset{+}{(-)}$
7000	0	$\overset{(+)}{-}$	—	0	0
1000	0	—	—	0	0

IV. DISCUSSION.

Although certain small morphological differences seem to be present between the c-mitosis of colchicine and that of *iso*-colchicine, the same main type undoubtedly prevails in both substances. On the other hand, the differences in threshold values are striking: in *Allium* colchicine gives c-tumours and c-mitoses even in concentrations a little above 100×10^{-6} mol/l, *iso*-colchicine gives tendencies to c-mitosis in 7000 and full effect in 14000×10^{-6} mol/l. The threshold is heightened 70 to 100 times in the *iso*-form.

LEVAN and ÖSTERGREN (l. c.), studying the c-mitosis of several hydrocarbons, found that, especially within related series, the activity thresholds are negatively correlated to the water solubility of the substances. The conclusion was drawn that the primary action on the cell was exserted physically and not chemically. It was stressed, however, that colchicine occupied an exceptional position among the c-mitotic substances owing to its high activity and high water solubility. GAVAUDAN, DODÉ and POUSSEL (1944), working partly on the material of LEVAN and ÖSTERGREN and partly on their own material, sharpened the opposition between, on one hand, colchicine and a few other readily soluble substances, and, on the other hand, the hydrocarbons and substances of low solubility. They put the c-mitotic action in relation to the thermodynamic activity of the c-mitotic substances.

This activity may be calculated with sufficient accuracy as the relation between the active concentration and the concentration of saturated water solution. To FERGUSON (1939) falls the merit of having introduced this entity in biology. By calculating the thermodynamic activity of certain narcotics, poisons, insecticides and bactericides he found that the substances generally fell into two well-defined groups: (1) The activity is directly correlated to physical properties of the substances, such as solubility or vapour pressure. In this group the threshold seldom lies below 0,1 of the water solubility, and since it cannot exceed 1 the thermodynamic activity varies very little, although the absolute values of the active concentrations may vary several thousand times. FERGUSON considered the effect of this type of substances to be of an unspecific, physical nature. (2) Great differences occur between activity threshold and water solubility. The thermodynamic activity in this group consequently varies a great deal; in highly soluble substances it is low, often of the size order of 0,001 or less. Here FERGUSON assumed the biological activity to be due to a more specific, chemical action. FERGUSON pointed out the possibility and gave instances of substances which take an intermediate position, acting at the same time physically and chemically.

If we apply FERGUSON's ideas upon colchicine and *iso*-colchicine some interesting features come to light. We have the following values (concerning the solubility of colchicine we must refer to a later paper):

	Solubility in 10^{-6} mol/l	Activity threshold in 10^{-6} mol/l	Thermodynamic activity
colchicine	> 500·000	150	< 0,000·3
iso-colchicine	50·000	14·000	0,28

It is immediately seen that colchicine and *iso*-colchicine belong to different types of substances: colchicine with its low thermodynamic activity is a typical representative of the chemically acting substances, while *iso*-colchicine with its 900 times higher thermodynamic activity belongs to the type of unspecifically acting substances. The most surprising result of the present investigation is, in fact, that such a small change in the colchicine molecule as the change from colchicine to *iso*-colchicine will so totally change the activity. Evidently, at least in the case of *Allium*, the specific or chemical activity of colchicine has been removed: for *iso*-colchicine there remains not much more than its physical activity.

In the introduction we referred to studies dealing with the question of what part of the colchicine molecule may be responsible for its

activity. Thus, BRUES and COHEN (1936) found that colchiceine (IV) or (VI) (Scheme 1) only had $^1/_{30}$ to $^1/_{40}$ of the activity of colchicine. The activity of N-acetyl-colchinol (VII) was similar. Its methyl ether (VIII) had only $^1/_{400}$ of the activity of colchicine. By testing various chemical compounds on animal material LETTRÉ and collaborators (l. c.) arrived at the conclusion that α-phenyl-β-(p-methoxy-phenyl)-ethylamine (IX) was the simplest »mitosis poison» of »colchicine type». By the construction of the new colchicine formula with seven-rings this conclusion has lost somewhat in significance. Moreover, it is now a well-known fact that much simpler substances with six-rings or simple aliphatic substances have c-mitotic activity. ÖSTERGREN (1944), for instance, enumerates about 80 organic substances, all of which exhibit c-mitotic activity more or less plainly (or in some cases latently).

What answer may be given to the question as to which group of a chemical compound is the active one? By studying the thermodynamic activity of various derivatives of a substance it may be possible to decide what groups only participate in the unspecific physical activity and what groups are necessary for giving a specific chemical effect. In our case it turned out that colchicine on being transformed into *iso*-colchicine lost its specific activity. The arrangement of keto- and methoxyl-groups as in ring *C* of colchicine must consequently be necessary for its specific action on *Allium* roots. To what extent the other groups of the molecule also take part in the specific activity, and how far our results in *Allium* may be valid in other materials, will be discussed in a later paper of the present series.

SUMMARY.

The c-mitotic activity of *iso*-colchicine is tested. It is found that a concentration of 14000×10^{-6} mol/l induces full c-mitosis and c-tumours. 7000×10^{-6} mol/l only giving tendencies to c-mitosis and no c-tumours. Its activity is thus about 100 times lower than that of colchicine.

FERGUSON's division of substances according to their thermodynamic activity into two groups, viz. unspecifically or physically active and specifically or chemically active, is adapted to the present case. It is claimed that colchicine has a specific activity, which gets lost on its transformation into *iso*-colchicine, this latter acting by its physical activity.

Svalöf, January, 1947.

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SOME OBSERVATIONS ON POLLINATION AND FRUIT SETTING IN ECUADORIAN CACAO

BY *ARNE MÜNTZING*
INSTITUTE OF GENETICS, LUND, SWEDEN

DURING a short stay at the Swedish Hacienda Clementina in the neighbourhood of Babahoyo, Ecuador, I had an opportunity to make some observations and experiments concerning pollination and fruit setting in *Theobroma cacao*. Four trees growing close to the hacienda and numbered 2, 3, 4 and 5 were used for the experiments. The origin of these trees is not quite clear, but they are most probably derived from crosses between »Cacao Nacional», the widespread native cacao variety of Ecuador, and trees raised from seeds imported from Venezuela.

A total of 156 flowers were pollinated, 81 with pollen from the same tree, 75 with pollen from other trees. On account of the structure of the flowers, which normally prevents spontaneous self-fertilization, it was not necessary to emasculate the flowers. However, before pollination the virginity of the stigmata was controlled with the help of a strong lens. Only flowers newly opened were used, and these were pollinated in the morning or some time before noon.

Results of self-pollinations. — On trees Nos. 2, 3 and 4 all self-pollinated flowers were abscissed and fell to the ground before the end of the 3rd day. The numbers of pollinated flowers were 14, 29 and 13 respectively. A few of these flowers, however, were used for fixation for embryological studies before the flowers had yet fallen. Nevertheless the pollinations clearly show that these three trees are self-incompatible. The fourth tree, No. 5, on the contrary, was found to be self-compatible. Of 13 self-pollinated flowers, which were allowed to develop up to 12 days before they were fixed, 11 showed an obvious fruit setting and an even and regular development. Twelve additional self-pollinated flowers were fixed within 3 days, before it was possible to decide whether the pollination had been successful. However, all ovaries in these flowers when observed more than 24 hours after pollination showed an obvious swelling, indicating a positive result also

in these cases. Though the degree of flowering in this tree was rather sparse, fruit setting was unusually rich (Fig. 1), all stages of young fruits being represented.

Of the self-incompatible trees especially No. 3 showed a very profuse flowering, but evidently only a small fraction of these flowers gave rise to fruits. In contrast to the self-compatible tree (No. 5) young developing fruits were quite sparse.

Fig. 1. The self-compatible tree (No. 5), showing a high degree of fruit setting.

Results of cross pollinations. — Part of the cross pollinated flowers were used for fixations before the seed setting could be judged. The remaining flowers gave the following result:

Cross combination	Flowers abscissed	Commencing fruit development
3 × 5	11	4
5 × 3	7	3
3 × 2	12	4
2 × 3	3	2
3 × 4	11	—

According to these data, a commencing fruit development was obtained in the cross self-incompatible × self-compatible (3 × 5) and reciprocally (5 × 3). The same result was obtained in one of the crosses self-incompatible × self-incompatible (3 × 2 and 2 × 3), whereas the other cross of this kind (3 × 4) gave a quite negative result. Thus, though quite preliminary the self and cross pollinations undertaken are sufficient to demonstrate that the cacao trees at Clementina are different as regards the conditions of fertilization and fruit development. Further information may perhaps be obtained from an embryological study of the material fixed. This work is carried out by my colleague, Dr. A. HÅKANSSON.

The degree of spontaneous pollination. — On the self-incompatible tree No. 3, which flowered abundantly, aphides and ants were present in large numbers. In order to study the possible rôle of the aphides for pollination the frequencies of pollen grains at the styles of lousy and non-lousy flowers were compared. The flowers were collected at 5 o'clock p. m., all open flowers being taken at random. Most of these flowers had opened in the morning of the same day, but some flowers were 2 or even 3 days old. After picking, the flowers were immediately divided into two categories, i. e. flowers with and without aphides on the flowers or flower-stalks. After staining over night in a mixture of aceto-carmine and glycerine in equal parts the styles were examined under the microscope. At first dry styles were used, but staining was found to be necessary in order to distinguish the pollen grains with certainty from the small papillae which occur at the style, especially at its basal part.

When the styles are inclosed in the aceto-carmine-glycerine under pressure of the cover slips, some pollen grains are detached from the stigmata and swim free in the medium. In order to eliminate this source of error the whole slides were carefully looked through and the number of free pollen grains counted.

The five stigmatal lobes of the style were often in close contact and pressed against each other even in flowers one day old, and in such cases it was sometimes difficult to decide wether the pollen grains were situated at the stigma or at the style below the stigma. The values given below represent the total number of pollen grains observed, whether situated at the stigma or lower down at the style. A few times pollen grains situated at the side of the style and even near the base were observed to have germinated and to have produced pollen tubes.

Whether these parts of the style are really equally conceptible as the stigma lobes remains to be studied.

The observations made may be summarized as follows:

Category	Number of styles	Number of styles with pollen	Number of pollen grains	Number of pollen grains per pollinated style	Number of pollen grains per all styles
Non-lousy flowers: ..	44	14	59	4,2	1,3
Lousy » : ..	58	37	485	13,1	8,4

Of the 44 non-lousy flowers only 14 were pollinated. Of the 58 lousy flowers, on the contrary, as many as 37 had pollen. Thus, these figures clearly demonstrate that the lousy flowers were better pollinated than the non-lousy ones. The two distributions 30 : 14 and 21 : 37 are significantly different, a χ^2-test giving a P smaller than 0,001.

The pollen counts also demonstrate that the frequency of unpollinated flowers is very high, and that the number of pollen grains at the pollinated flowers is as a rule insufficient to give fruits with a normal number of seed. The seed number per fruit was counted in four different populations of the hacienda Clementina. One ripe fruit was taken at random from each tree, the number of trees examined in this way ranging from 43 to 75. The following result was obtained:

	Number of seeds per fruit											
Population	5 - 10 - 15 - 20 - 25 - 30 - 35 - 40 - 45 - 50 - 55										n	M
La Victoria		2	3	1	3	8	13	7	6		43	35,15
Sta Isabel	1	2	5	6	9	15	12	14	3	1	68	32,80
San Rafael	1	1	3	4	9	5	12	28	17	5	85	38,60
Nacional		2	6	7	5	10	17	18	8	2	75	35,15

Evidently the number of seeds per fruit is rather variable, the individual values ranging from 5 to 55 with average values from 32,80 to 38,60. The distributions in each series show a rather pronounced skewness, the maximum frequency class being rather close to the class representing the highest seed number.

The variation in seed number per fruit may be due to three causes, (1) a variable number of functional ovules, (2) a variation in the number of ovules which are fertilized, and (3) a variation in the number of fertilized ovules which develop normally. The pronounced skewness of the curves strongly indicates that the second cause is the most important factor. The number of functional ovules is probably close to the maximal number of seeds, but if pollination is insufficient a variable number of these ovules will not be fertilized.

According to the values given above, 51 flowers (14 + 37) had been pollinated, the average number of pollen grains per pollinated style being 4,2 in the non-lousy flowers and 13,1 in the lousy ones. It is of interest to analyse the distribution of the pollen grains on the different styles. The following values were obtained:

Slide No.	Number of pollen grains at the stigma or style								Number of free grains
	1 — 5	— 10	— 15	— 20	— 25	— 30	... 70	— 80	
1 (lousy)	5	2	—	1					4
2 »	8	1	—	1	1				72
3 »	3	—	3	—	—	—	—	1	36
4 »	7	1	1	1	—	1			76
5 (non-lousy) ...	2	—	1						33
6 » ...	4	—	1						0
7 » ...	2								0
8 » ...	3	1							3
Total	34	5	6	3	1	1	—	1	224

Of the 51 styles observed to be pollinated only one had with certainty received a surplus of pollen (more than 70 grains). A few of the other ones had also received a sufficient number of pollen grains for fruit development but probably not for the maximal or even the average number of seeds.

Though the presence of free pollen grains, being detached from the styles, is a disturbing source of error the counts nevertheless demonstrate that the majority of the pollinated flowers had received less than 5 pollen grains. This figure also includes grains not situated at the stigma but at other parts of the style. Such a low number of pollen grains may perhaps be able to start fruit development, but only in quite rare cases will this result in a ripe fruit.

In cacao it is a regular phenomenon that a considerable proportion of the young fruits stop development a few weeks after pollination and die when they have reached a length of a few cm. This cherelle wilting seems to be a normal physiological phenomenon caused by the limited ability of the tree to bear fruit.

HUMPHRIES (1941) has shown that cherelle wilt is primarily due to competition between pods for water and nutrients. Considering the present observations, which show that most of the pollinated styles receive a quite insufficient number of pollen grains, it is tempting to assume that cherelle wilting is largely caused by incomplete pollination.

POUND (1931 a), however, tested this possibility and did not find any difference in the number of developing ovules in wilting and normal cherelles having a length of two inches. On the other hand, POUND also observed that in ovaries examined about 6 days after pollination some of the ovules had hardly grown at all. The proportion of ovules which fail at this time varies considerably and may reach 50 per cent or even higher. The number which survives presumably bears a close relation to the number of beans in the mature pod. POUND points out that the ovules which fail do so either because they have not been fertilized or because the union is such that the resulting zygote dies. POUND (1931 b) also found a marked variation in bean number in mature pods. In POUND's material the frequency curves of bean number per pod also showed a marked asymmetry, the deviations below the modal number being far more numerous than those above. This suggested incomplete development of potential seeds and led to enquiry into the variability of ovule number per ovary. This number was found to be much less variable and on an average higher than the bean number per pod. This result is evidently in perfect agreement with the conclusion drawn from my own observations, viz. that the variation in bean number is largely due to incomplete pollination. A contributing cause may be inviability of certain zygotic combinations. The latter possibility might occur if a flower happens to be pollinated with a mixture of pollen, part of which gives compatible zygotic combinations, the other part incompatible combinations.

The whole question of seed setting and cherelle wilting in cacao must be considered in the light of similar problems in orchard plants. A review of the observations gathered on the causes of fruit dropping in apples, pears, plums and other orchard plants is given by BRINK and COOPER (1941) in a paper on incomplete seed failure as a result of somatoplastic sterility. In this review it is pointed out that in some cases at least the first fruit-drops represent fruits with unpollinated ovules, the later drops fruits in which embryo development has ceased or is abnormal. BRINK and COOPER point out that this failure probably represents cases of somatoplastic sterility, i. e. a disturbed balance between the endosperm and the maternal tissues.

That aphides contribute to the pollination of the cacao flowers has already been shown by HARLAND (1925) and STAHEL (1928), who observed the amount of pod setting in the presence or absence of various insects. STAHEL concludes that pollination is accomplished by ants which tend the aphides and also, apparently, by various small flying

insects, since wind pollination apparently does not occur. POSNETTE (1944) reviews the whole problem of cacao pollination, especially with reference to the conditions in Trinidad. According to this author, the most important contribution to the extensive literature on the pollination of cacao flowers was made by BILLES (1941) when he drew attention to the Ceratopogonid midge, *Forcipomyia* sp. Subsequent investigation has shown that this insect is probably the most important pollinator and therefore directly responsible for the bulk of the cacao crop in Trinidad.

At the hacienda Clementina in Ecuador I did not observe any larger flying insects in the cacao flowers than thrips. The results described above demonstrate that the aphides, directly or indirectly, through the action of the accompanying ants, contribute to the pollination. This pollination is probably of value for the self-compatible trees and may help to increase the amount of seed setting in such individuals as are now known also to occur in Ecuadorian cacao populations. For seed setting in the self-incompatible trees, on the contrary, the aphides are probably of no value, unless the tending ants occasionally run from one tree to another.

The problem of self-incompatibility in cacao is especially interesting from a theoretical point of view, as incompatibility in this species is not due to a checked pollen tube growth but to a disturbed development after fertilization. Another problem is represented by the fact that in Trinidad self-incompatible trees are also incompatible with each other and only set seed when receiving pollen from a self-compatible tree. In other parts of South America, especially the Amazon region, self-incompatible trees may be compatible with each other. Gradations in the degree of compatibility and incompatibility have also been observed. Incompatibility seems to be more pronounced between individuals within a population than between individuals belonging to different populations. All these facts have chiefly been worked out by the cacao specialists at the Imperial College of Tropical Agriculture, Trinidad, British W. I., and the literature on this problem is already extensive. References to the various publications on this subject by POUND, VOELCKER, COPE, POSNETTE and others are given in a recent paper by POSNETTE (1945). This paper is also of special interest as it deals with pollination results obtained with Amazon material, including material introduced from Ecuador. All plants of the latter kind were found to be self-incompatible, but some combinations between self-incompatibles gave a positive result whereas other combinations failed. The Ecuadorian types were similar in this respect to the other material from the

Amazon region and differed from the Trinitario cacao, in which self-incompatibles cannot intercross successfully.

In my own crosses similar results were obtained, apparently not all self-incompatible trees being incompatible with each other. In contrast to the Ecuadorian types studied by POSNETTE my own material also included a self-fertile tree. Such trees are highly important not only for the seed setting of the self-incompatible trees but also on account of their yield, which, on an average, is higher than the yield of self-incompatible trees. Now that the breeding of new disease-resistant and high-yielding cacao varieties must be based on clonal reproduction (cf. CHEESMAN, 1946), it is necessary to have a detailed knowledge of the conditions of fruit setting in these clones. More basic research is evidently needed before the conditions of pollination and seed production in cacao can be fully understood and controlled.

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STUDIES ON LINKAGE RELATIONS OF THE Cy FACTORS IN PISUM

BY *ROBERT LAMM*

THE STATE HORTICULTURAL RESEARCH STATION, ALNARP, ÅKARP, SWEDEN

INTRODUCTION.

BESIDES the major factor *Le* for long internodes in *Pisum sativum* L. a number of modifiers are known. Among these the author (LAMM, 1937) has especially studied the polygenic factors Cy_1 and Cy_2. The *Cy* factors are suppressors, which as dominant to a certain degree inhibit the growth in length of the internodes of dwarf peas, i. e. peas recessive for *le*. Of Cy_1 only one recessive allele is known, whereas the locus Cy_2 appears to carry the two mutant genes cy_2^c and cy_2^s. Plants of the genotype *le le* cy_1cy_1 $cy_2^ccy_2^c$ are phenotypically so-called crypto-dwarfs, which more especially at the juvenile stage have longer internodes than the common dwarfs. In the slender type (*le le* cy_1cy_1 $cy_2^scy_2^s$) the internodal length at any stage is much more elongated than in common dwarfs and crypto-dwarfs. More complete descriptions of these types have been given by RASMUSSON (1927) and DE HAAN (1927, 1930). In the literature the following symbols have been used for the *Cy* factors.

RASMUSSON 1927	DE HAAN 1927, 1930	LAMM 1937	V. ROSEN 1944
Cry_1	*La*	Cy_1	—
cry_1	*la*	cy_1	—
Cry_2	*Lb*	Cy_2	—
cry_2	—	cy_2^c	—
—	*lb*	cy_2^s	lb^s

The paper of RASMUSSON mentioned above was published somewhat earlier in 1927 than that of DE HAAN. In order to co-ordinate the symbols for this group of length factors I decided (LAMM, 1937) to call them »*cy*» with indices. The scope of my recent work has been to find out their linkage relations. I have also tried to find evidence for the correctness of DE HAAN's suggestion of the possible existence of a duplication in *Pisum* (DE HAAN, 1932).

MATERIAL.

Accurate information as to the origin and genotype of the pure lines used in *Pisum* investigations are highly desirable. Since structural hybridity is probably not rare, it might be of importance to know the relations between the lines used by various authors. A survey of the lines used for the present investigation is given in Table 1.

RESULTS OF CROSSES AND THEIR STATISTICAL ANALYSIS.

In order to study the linkage relations of the *Cy* factors a number of crosses have been performed. Table 2 gives a survey of the bifactorial segregation numbers collected from F_2 counts employed in the linkage studies. The five first columns of this table contain some general information such as the parental lines, the factors concerned, the phase of crossing (C = coupling, R = repulsion), and the percentage of seed germinated. In this connection it should be added that the pollen-fertility of the F_1 plants has regularly been investigated. No case of total or partial male sterility has been detected. In the next five columns of Table 2 the total number of plants and the observed number of plants of different categories per sixteen are given. If desired, the reader may thus readily compute the absolute number of plants of various categories and also recalculate the χ^2 analysis given in the following columns of the table. This χ^2 analysis has been performed in accordance with methods given by MATHER (1938, Chapter IV). In cases of significant single factor deviation, χ^2 for linkage has been computed according to the special formula given by MATHER, 1938, p. 89. The heterogeneity between crosses and reciprocals has also been computed. With but one exception no significant heterogeneity has been revealed, and therefore χ^2 values for heterogeneity have not been given in Table 2. Significance has been marked with asterisks, one, two and three asterisks corresponding to respectively $P = 0{,}05—0{,}01$, $P = 0{,}01—0{,}001$ and $P < 0{,}001$. Having detected the presence of linkage for two genes further estimations have been made according to the method of maximum likelihood (cf. MATHER, 1938, Chapter V).

THE LINKAGE RELATIONS OF Cy_1.

The linkage between Cy_1 and *St* has been studied in the crosses 43/1, 43/2 and 43/6 of Table 2. χ^2 for linkage is highly significant. In case of absolute linkage these crosses performed in the repulsion phase

TABLE 1. *Survey of the pure lines used for the present investigation.*

Line No.	Name (within parenthesis, see footnote)	Origin and references	Marker genes used by the author											
			Stem			Leaf				Flowers		Pod		Seed	
1	Frühe niedrige	NILSSON, 1936, L. 76	*le*	*cy*$_1$	*Cy*$_2$	*Wa*	*Wb*	*Wlo*	*St*	*a*	—	*N*	*Gp*	*R*	*I*
2	Graue niedrige	RASMUSSON, 1927, *Gd*	»	»	»	»	»	»	»	*A*	*B*	»	»	»	»
3	201 . 1	DE HAAN, 1927	»	»	»	»	»	»	»	»	»	»	»	»	»
4	Witham wonder	RASMUSSON, 1927, *Ww*	»	*Cy*$_1$	*cy*$_2$^{c}	»	»	»	»	*a*	—	»	»	*r*	*i*
5	English wonder mut.	NILSSON, 1933, L. 89	»	»	»	»	»	*wlo*	»	»	—	»	»	»	»
6	204 . 1	DE HAAN, 1927	»	»	*cy*$_2$^{s}	*wa*	»	*Wlo*	»	»	—	»	»	*R*	»
7	Acacia from Vilmorin	NILSSON, 1933, L. 54	»	»	»	*Wa*	»	»	»	»	—	»	»	*r*	*I*
8e	Crypto-dwarf (from 2 × 4)	RASMUSSON, 1927	»	*cy*$_1$	*cy*$_2$^{c}	»	»	»	»	»	—	»	»	*R*	*i*
9a	» (from 1 × 5)	NILSSON, unpubl.	»	»	»	»	»	*wlo*	»	*a*	—	»	»	*r*	*I*
9b	» (from 1 × 5)	»	»	»	»	»	»	»	»	»	—	»	»	—	*i*
10	Slender (from 3 × 6)	DE HAAN, 1927	»	»	*cy*$_2$^{s}	*wa*	»	*Wlo*	»	*A*	*B*	»	»	*R*	»
11	» (from 7 × 1)	NILSSON, 1934	»	»	»	*Wa*	»	»	»	*a*	—	»	»	»	*I*
13a	» (from 9a × 6)	LAMM, 1937, cross 43	»	»	»	»	»	*wlo*	»	»	—	»	»	»	»
13b	» (from 9a × 6)	» , » , »	»	»	»	*wa*	»	»	»	»	—	»	»	*r*	*i*
14b	» (from 9a × 11)	» , » , cross 54	»	»	»	*Wa*	»	»	»	»	—	»	»	*R*	*I*
15	» (from 2 × 10)	» , » , » 18	»	»	»	»	»	*Wlo*	»	*A*	*B*	»	»	»	»
16a	» (from 13a × 22)	» , unpubl.	»	»	»	»	»	*wlo*	»	*a*	—	*n*	»	*r*	»
16c	» (from 13a × 22)	» , »	»	»	»	»	»	»	»	»	—	»	»	*R*	—
18	Witham wonder mut.	HYLMÖ, unpubl.	»	*Cy*$_1$	*cy*$_2$^{c}	»	»	*Wlo*	»	»	—	*N*	»	*r*	*i*
22	Olympia W:s/41	» , 1943	»	»	»	»	»	»	»	»	—	*n*	»	»	»
25c	Dwarf (from 18 × 41)	LAMM, unpubl.	»	»	»	»	*wb*	»	»	»	*b*	*N*	»	»	»
30	» (from 48 × 14b)	» , »	»	»	*cy*$_2$^{s}	»	*Wb*	*wlo*	*st*	»	—	»	»	*R*	*I*
40a	Emerald gem	NILSSON, 1933, L. 43	*Le*	—	—	*wa*	»	*Wlo*	*St*	»	—	»	»	»	»
41	Chenille-a	» , » , L. 88	»	—	—	*Wa*	*wb*	»	»	»	—	»	»	»	»
42	Chenille-A	» , » , L. 85	»	—	—	»	»	»	»	*A*	*B*	»	»	»	*i*
44	Goldfähnchen	» , unpubl. L. 136	»	—	—	»	*Wb*	»	»	*a*	—	»	*gp*	*R*	*I*
48	De Winton	v. ROSEN, 1944, No. X	»	*Cy*$_1$	—	»	*wb*	»	*st*	*A*	*b*	»	*Gp*	»	»

The pure lines from which the crosses in question have been obtained are given within the parenthesis. These pure lines can be found in the first column of the table. The names of the makers of these crosses are given in the column headed »Origin and references».

TABLE 2. *F_2 analysis of bifactorial crosses.*

Cross No.	Parental lines	Factors	Phase	% germ.	Total No. of plants	Observed number of plants per 16				χ^2 first factor	χ^2 second factor	χ^2 linkage
						AB	Ab	aB	ab			
43/1	30 × 13a	*Cy_1St*	*R*	94	163	7,56	4,71	3,73	—	0,247	1,720	22,332***
43/2	»	»	»	98	130	8,98	3,82	3,20	—	1,733	0,092	8,209***
Pooled ...	»	»	»	97	293	8,19	4,31	3,49	—	1,557	0,602	29,519***
43/6	16a × 30	»	»	98	488	7,34	4,56	4,10	—	0,098	3,158	73,457***
Total		»	»	98	781	7,66	4,47	3,87	—	0,267	3,534	102,064***
42/3	25c × 15	*Cy_1B*	*R*	99	151	8,69	4,13	2,75	0,42	2,121	0,974	4,363*
40/7	13b × 1	*Cy_2Wa*	*C*	91	157	12,23	0,10	0,20	3,46	0,359	0,614	123,064***
37/17	9b × 2	*Cy_2Wlo*	*C*	91	440	8,84	2,84	3,27	1,05	0,982	0,109	0,000
38/4	5 × 6	*Wa Wlo*	*R*	92	479	8,78	2,97	3,11	1,14	0,585	0,118	0,123
33/34	11 × 4	*Cy_2R*	*R*	95	125	8,96	3,58	2,56	0,90	0,771	0,600	0,108
33/34	11 × 4	*Cy_2I*	*R*	95	125	9,47	3,07	2,69	0,77	0,771	0,067	0,044
33/54	9b × 11	»	»	90	166	8,77	3,47	2,80	0,96	0,201	0,651	0,131
Total		»	»	92	291	9,07	3,30	2,75	0,88	0,835	0,194	0,168
33/7	8c × 1	*Cy_2A*	*R*	97	220	8,65	3,49	2,47	1,38	0,097	3,491	0,978
33/9	10 × 1	»	»	98	162	9,38	2,77	3,36	0,49	0,074	1,852	1,451
Total		»	»	97	382	8,96	3,18	2,85	1,01	0,171	0,283	0,001
37/18	9a × 2	*Cy_2*A	*C*	99	160	9,70	2,80	2,70	0,80	0,833	0,533	0,011
37/19	11 × 2	»	»	99	283	8,82	2,54	3,11	1,53	2,385	0,029	3,398
Total		»	»	99	443	9,14	2,64	2,96	1,26	0,470	0,091	2,661
42/11	16c × 2	*Cy_2N*	*C*	76	100	10,24	3,04	2,08	0,64	3,413	0,213	0,003
42/15	2 × 16c	»	»	72	234	10,19	3,69	1,57	0,55	17,236***	0,279	0,009
Total		»	»	74	334	10,20	3,50	1,72	0,55	20,124***	0,036	0,006
40/5	22 × 6	*WaN*	*R*	87	146	9,97	2,30	3,29	0,44	0,228	4,831*	0,897
44/79	6 × 44	*Wa Gp*	*R*	62	90	8,18	4,09	3,20	0,53	0,133	0,726	3,086
44/84	44 × 6	»	»	73	106	8,91	2,72	3,62	0,75	0,314	0,616	0,507
Total		»	»	68	196	8,57	3,35	3,43	0,65	0,027	0,000	2,939

would give the segregation 2 : 1 : 1 : 0. The χ^2 analysis actually shows a very good agreement with this assumption, and the following non-significant χ^2 values, each having two degrees of freedom, have been obtained: χ^2 for deviation from expectation = 3,5481; for differences between reciprocals = 2,2370 and for heterogeneity between crosses = 2,3747. In this case of strong linkage detected in repulsion crosses it would, however, be unwise not to extend the analysis to the F_3.

For the F_3 analysis seed was taken from the dwarf F_2 plants with normal stipules (category AB of Table 2). Slender F_2 plants were less suitable because of a somewhat reduced seed setting. As shown in Table 3, no fewer than 374 F_3 families were investigated. In order to save space the maximum number of seed sown per family was restricted to 60. Altogether 59 families, each giving less than 16 plants, were rejected from the calculations. With this restriction of family size misleading results due to faulty classification should not occur in more than one per cent of cases (cf. MATHER, 1938, pp. 26—27) provided absolute linkage and non-disturbed segregations also characterized these families. In these remaining 315 families of more than sixteen plants germination varied between 27 and 100 per cent, and was especially low in the F_3 progenies of the cross No. 43/6. These circumstances have also been taken into consideration. Obviously segregation values must be cautiously judged in case of bad germination, since there may exist correlation between the germinability and the occurrence of certain of the segregating genotypes. The 2 : 1 : 1 segregating F_3 families (category »*m*» of Table 3) were chosen for such an investigation, the result of which has been given in the analysis following Table 3.

TABLE 3. *Investigation of F_3 families derived from F_2 plants of the AB type.*

Symbols F_2 genotype	Nos. of fam. (> 16) classified into the categories below					Sum	Not classified (< 16)	Total No. of fam.
	i	*j*	*k*	*l*	*m*	*n*		
	Cy_1St » »	Cy_1St cy_1 »	Cy_1St » *st*	Cy_1St cy_1st	Cy_1st cy_1St			
Cross No. 43/1 ...	—	2	1	—	72	75	2	77
» » 43/2...	—	3	2	1	63	69	4	73
» » 43/6...	—	4	4	—	163	171	53	224
Observed total	—	9	7	1	298	315	59	374
Exp. $p = 0{,}0282$	0,1	8,6	8,6	0,3	297,4	315,0		

Analysis of certain possible correlations in the »m» families of Table 3. — These families are characterized by 2 : 1 : 1 segregation into plants of the types AB, Ab and aB.

Cross No.	43/1	43/2	43/6
Nos. of families	72	63	163
Mean number of plants per family	42,6	50,5	34.5
Average percentage of germination	72,4	86,5	59,9
» » » Cy_1 *st* plants	26,8	25,0	24,4
» » » cy_1 *St* »	24,2	24,1	26,8
Correlation coeff. (r) between:			
% germinated and % Cy_1 *st*	— 0,192	— 0,016	+ 0,147
» » » » cy_1 *St*	— 0,140	+ 0,133	— 0,087

None of these correlation coefficients are significant.

The above analysis indicates that in spite of the relatively poor germination the risk of misleading classification of the F_3 families is probably fairly small. The occurrence of families of the categories *j*, *k* and *l* in Table 3 shows that the linkage between Cy_1 and *St* is not absolute. The ratio between such 3 : 1 segregating families and the 2 : 1 : 1 segregating families of the *m* category has been analysed by the χ^2 method, with the result that no heterogeneity between crosses could be detected (χ^2 for heterogeneity = 1,93). For the estimation of the linkage between Cy_1 and *St* I have therefore used the observed totals of Table 3. The method of maximum likelihood has also been applied here (cf. MATHER, 1936). Combination of the F_2 and F_3 data discussed in the pages just cited has been avoided, since the present case is that of a recombination value from a close repulsion F_2 (cf. MATHER, 1935, p. 401).

The recombination fraction *p* is given by maximizing the logarithm of the likelihood expression

$$\frac{dL}{dp} = i \log \frac{p^2}{2+p^2} + (j+k) \log \frac{2p(1-p)}{2+p^2} + l \log \frac{2p^2}{2+p^2} + m \frac{2(1-p)^2}{2+p^2},$$

which gives

$$2(2i + j + k + 2l) - 4np - (j + k + 2m)p^2 = 0.$$

Inserting the total values for *i*, *j*, *k*, *l*, *m* and *n* of Table 3, the solution of the above equation gives $p = 0{,}0282$. The standard error of *p* is obtained from the expression

$$sp = \sqrt{\frac{p(1-p)(2+p^2)^2}{4n(2+2p-p^2)}} = \pm 0{,}006508.$$

The crossing-over value between *Cy*$_1$ and *St* has thus been estimated at 2,82 ± 0,65 per cent.

It is now easy to calculate the expected segregation of the 781 F_2 plants representing the totals for crosses Nos. 43/1, 43/2 and 43/6 of Table 2. A comparison between expected and observed is given below.

Categories	AB	Ab	aB	ab	n
Observed	374	218	189	0	781
Expected ($p = 0{,}0282$)	390,7	195,1	195,1	0,2	781,1

χ^2 for deviation (categories aB and ab being pooled) amounts to the non-significant value 3,605.

Table 3 shows that 298 families of the 2 : 1 : 1 segregating category »*m*» have been investigated. These families were represented by no fewer than 11874 plants. It seems perhaps astonishing that not a single slender plant with reduced stipules (*cy*$_1$ *st*) has been observed among these plants. To answer this question we may calculate the minimum size of family required in order to obtain at least one individual of that category. Applying the detailed instructions given by MATHER (1938, pp. 26—29) with the maximum error 1 : 100 such a family should comprise about 22400 plants. If for some reasons crosses for the detection of linkage can only be made in the repulsion phase, the inference of absolute linkage must obviously be drawn with the greatest caution.

In his extensive investigations of linkage relations in *Pisum* LAMPRECHT (1946) found the average linkage value 27 per cent between *B* and *St*. Cross No. 42/3 of Table 2 shows that there is also, as should be expected, linkage between *Cy*$_1$ and *B*. The percentage of crossing-over amounts to 33,9 ± 7,10. Because of the high standard errors, the sequence of the genes *Cy*$_1$—*St*—*B* cannot be told with accuracy. Now plants are available, however, making it possible to perform new tests in the coupling phase.

Cross No. 42/3 was made between line No. 25 c and line No. 15. The complete F_2 segregation is given in the survey below, where the expected segregations as regards internodal length and flower colour are also inserted.

	Dwarf	Crypto-dwarf	Slender	Total	Expected (9:3:4)
Red	82	19	7	108	123,75
Pink	39	2	2	43	41,25
White	54	11	4	69	55,00
Total	175	32	13	220	—
Expected (12 : 3 : 1)	165,00	41,25	13,75	—	220,00

In neither case is the deviation from expectation significant ($\chi^2 = 5{,}643$ and 2,721 respectively). For the estimation of the linkage between Cy_1 and B crypto-dwarf and slender plants have been pooled and white plants have been excluded. The data concerning cross No. 42/3 thus obtained are given in Table 2.

THE LINKAGE RELATIONS OF Cy_2.

DE HAAN (1927, 1930) found absolute linkage between the length factor *Lb* (similar to Cy_2) and a factor *W* for the production and distribution of bloom. The recessive alleles of these factors were introduced into his crosses by the pure line No. 6. Now this line has further been reciprocally crossed by the author with line No. 40 a, which is recessive for the known wax-factor *Wa*. The F_1 and F_2 of these crosses were constantly emerald (non-waxy). The number of F_2 plants investigated was 147 in the cross No. 39/5 between lines Nos. 6 × 40 a and 463 in the reciprocal cross No. 39/6. The germinability was in both cases good, being 91 and 95 per cent respectively. These results show that the wax-factor of line No. 6 is identical with *wa*.

Provided the plants are dominant for the basic wax-gene *Bl* a necessary condition for the normal glaucous covering of their green parts is dominance in either *Wa*, *Wb* or in both of them (WELLENSIEK, 1928). This author has shown that recessivity of these genes results in a more or less complete reduction of the bloom, giving plants of emerald type. Of either gene WELLENSIEK claims to have found three multiple allelomorphs. His descriptions of the different genotypes are unfortunately somewhat incomplete, as pointed out by LAMPRECHT (1939), who gives good descriptions for the distribution of wax characterizing the plants of his own lines.

As regards the symbols for these wax-genes, I have followed the modifications introduced by NILSSON (1933), which have also been adopted by LAMPRECHT (1939). In the present investigation no attempts have been made to distinguish between the recessive allelomorphs within the *Wa*- and *Wb*-series. Line No. 6 as well as line No. 40 a were characterized by slightly glaucous stems in contrast to the non-waxy stipules and pods. The leaflets of the former line appeared to be more glaucous than those of the latter. WELLENSIEK (1928), however, points out that the amount of wax on the foliage does not demonstrate typical differences. In a cross between line No. 40 a and line No. 42 a beautiful 9 : 7 segregation was obtained with respect to the presence of bloom. This cross was made in order to control the genotype of the former line, and it verified a similar result earlier obtained by NILSSON (1933, p. 220). Line No. 42 was also crossed with line No. 6. As expected, the F_1 was glaucous and the F_2 segregated into glaucous and emerald. Unfortunately the proportions were not determined.

As mentioned earlier, DE HAAN considered the linkage between Cy_2 and the wax-factor, now known to be *Wa*, to be absolute. The results

of the particular crosses upon which this conclusion was based are given in Table 4, where a control cross made by the author is also inserted. In case of free combination the four categories of this table would have occurred in the ratio 45 : 15 : 3 : 1. The observed segregations are, however, in accordance with that expected for absolute linkage, i. e. 48 : 12 : 0 : 4 with the exception of the results obtained by DE HAAN (1927), where excess of dwarf, emerald plants contributes to a significant χ^2. In spite of this, neither the total deviation nor the heterogeneity is significant. In this connection it is perhaps of some interest

TABLE 4. *F_2 analysis of crosses between line 6 and 3.* *($Cy_1cy_2{}^swa \times cy_1Cy_2Wa.$)*

Results obtained by	Number of plants classified as: dwarf glaucous	dwarf emerald	slender glaucous	slender emerald	Total No. of plants	χ^2 for dev.
DE HAAN, 1927	423	137	—	39	599	7,026*
» » , 1930	5426	1417	—	439	7282	2,754
LAMM, 1942	320	79	—	21	420	1,130
Total observed	6169	1633	—	499	8301	
Exp. 48 : 12 : 0 : 4 ($p = 0$)	6225,8	1556,4	—	518,8	8301,0	
χ^2 for deviation	0,517	3,766	—	0,757		5,040
» » heterog.						5,870
Exp. $p = 0{,}0199$	6205,3	1576,9	20,44	498,4	8301,0	
χ^2 for deviation	0,212	1,997	20,440	0,001		22,650***

to point out that in progenies segregating for *Wb* the recessives, according to v. ROSEN (1944, p. 272), are in excess.

The linkage between Cy_2 and *Wa* has also been studied in cross No. 40/7 (of Table 2) performed between the slender line 13 b and the dwarf line No. 1. This slender line in turn has been extracted from the progeny of a cross between lines 9 a and 6. The recessives are thus of the same origin as in the crosses of DE HAAN, though the genotypical background is different. Quite against the assumption of absolute linkage three recombination plants appeared among 157 F_2 plants. The crossing-over percentage as determined by the method of maximum likelihood amounts to $1{,}99 \pm 1{,}13$. The two bottom rows of Table 4 show that the results of cross No. 40/7 are significantly different from the cases of seemingly absolute linkage previously discussed. If this disagreement is due to real differences in the frequencies of crossing-over (i. e. genetically changed linkage values) or if they are caused by

differences in the viability of certain genotypes or other circumstances cannot be determined from the present investigations. Anyhow, the earlier assumption of absolute linkage between *Cy*$_2$ and *Wa* must not be generalized.

In this connection it should also be pointed out that the description of the slender phenotype given by DE HAAN (1930) in certain details is only valid for his own lines. This author observed a rather reduced fertility with regard to the seed content of the pods. Frequently, seedless pods were formed. Among slender lines of other origin, e. g. among those which I obtained from NILSSON (cf. LAMM, 1937), seed setting was much more abundant although still reduced as compared with normal dwarfs. It does not seem quite impossible that there might be an interaction between the viability of certain genotypes and the degree of seed setting.

In my earlier paper (LAMM, 1937) I suggested that *Cy*$_2$ was linked with *Wlo* (for the description of this gene, see NILSSON, 1933). Unfortunately this was definitely a mistake due to insufficient knowledge of the material at that time. The slender plants were studied at a rather young stage, and their relatively tender leaflets were misclassified as belonging to the *wlo* type. More recent studies have shown that there is no indication of linkage between *Cy*$_2$ and *Wlo* or *Wa* and *Wlo*, as can be seen from cross Nos. 37/17 and 38/4 of Table 2.

In the F_2 progeny of the cross last mentioned an unexpected segregation of the length of the young plants was observed. The same phenomenon of an unexpected length segregation has been noted in crosses between lines 6 and 18. That other factors than *Cy*$_1$ and *Cy*$_2$ also modify the length of dwarf peas is known from the investigations of DE HAAN (1930) and RASMUSSON (1938).

RASMUSSON (1927) and DE HAAN (1927, 1930) have shown independent segregation between *Cy*$_2$ and the two length factors *Cy*$_1$ and *Le*. WELLENSIEK (1928) states that there is no linkage between *Wa*, *Wb*, *R* and *I*. Since there is strong linkage between *Cy*$_2$ and *Wa*, the former factor cannot be coupled with any one of the three factors last mentioned. With respect to *R* and *I* the crosses Nos. 33/34 and 33/54 of Table 2 further support this statement. As regards the cross No. 33/34 segregating into 12 dwarf : 3 crypto-dwarf : 1 slender the linkage relations were determined within the two categories last mentioned.

In studies of small progenies v. ROSEN (1944, p. 300) found indications of independent segregation between *Cy*$_2$ and the genes *D*, *M* and *A*, whereas a possible linkage of 42 per cent might exist between *Cy*$_2$ and *Td*. He strongly points out that this rather unsure linkage relation must not be taken for granted. The crosses 33/7, 33/9, 37/18

and 37/19 of Table 2 are all concerned with the linkage relation between *Cy*$_2$ and *A*. A significant heterogeneity ($\chi^2 = 5{,}060$) has been found between the second factor segregation of the crosses Nos. 33/7 and 33/9. Estimations based on the totals of these two crosses therefore have little value and perhaps ought to have been omitted from the table. In the cross 37/19 there is a slight indication of linkage between *Cy*$_2$ and *A*, but there is no significance, and my own results together with those obtained by v. ROSEN (1944) support the statement of independent segregation.

In 15 : 1 segregating crosses with respect to dwarf and slender or crypto-dwarf, which crosses were at the same time heterozygous for *A*, the proportion of red : white among the crypto-dwarf and slender plants was 50 : 19. The expected proportion in case of independent segregation between *Cy*$_2$ and *A* is 51,75 : 17,25.

Td by LAMPRECHT (1945) was localized to a linkage group of the constitution *N*—15 %—*Z*—18 %—*Fa*—28 %—*Td*. If v. ROSEN's suggestion of a linkage between *Cy*$_2$ and *Td* corresponding to 42 per cent crossing-over is true, the former gene if situated to the left of *Td* must show linkage with *N*. The crosses 42/11, 42/15 and 40/5 of Table 2 refute this assumption. Two of these crosses are characterized by bad single factor segregations, but in spite of this the statement of independent segregation between *Cy*$_2$ and *N* seems to be valid. On the other hand, should *Cy*$_2$ happen to be situated to the right of *Td* the possible linkage between these two genes is still an open question.

The last but most interesting linkage relation to be discussed is that between *Wa* and *Gp* investigated by means of the two small progenies of the crosses 44/79 and 44/84 of Table 2. Unfortunately the germination power of the seed was bad, which contributes to the insecurity of the results. There is no heterogeneity between the two crosses. The single factor segregations of their totals are good, whereas χ^2 for linkage is somewhat high but not at all significant. In spite of this, linkage has been calculated by the method of maximum likelihood. The crossing-over value for the total of the two crosses thus obtained amounts to $39{,}9 \pm 5{,}92$ per cent.

I also tried to get a yellow-podded slender line in order to be able to test the linkage between *Cy*$_2$ and *Gp* in the coupling phase by crossing line No. 44 with the slender line No. 14 b. DE HAAN (1930) in crosses between high and slender obtained the segregation 45 high : 15 dwarf : 4 slender. Of cross No. 41/5 (line 44 × 14 b) 343 F_2 seeds were obtained, while 345 seeds resulted from the reciprocal cross No. 41/6. These seeds were sown, and respectively 21 and 14 slender plants were

obtained, all of which were rather weak and did not produce pods. The progenies also contained high and dwarf plants. This at present inexplicable behaviour of the exceptionally slender plants seems worthy of mention in this connection.

Future investigations of the possible linkage between Cy_2 and the genes of the *Gp* group are, however, very much needed. For if the crossing-over value of about 40 per cent obtained between *Wa* and *Gp* should turn out to be true, this would be attended by some interesting consequences, which will be discussed in the following chapter. The author, however, is anxious that the linkage suggested should not be taken for granted until new facts are available. The linkage of Cy_2 is thus still an open question.

DISCUSSION.

As mentioned in the introduction, I have tried to prove the existence of a duplication in *Pisum*. If a number of different polygenic (15 : 1 segregating) pairs of genes were distributed between two structurally and genetically similar linkage segments situated in two non-homologous chromosomes, this would be a strong indication of duplication. Genes that might be involved are, for instance, Cy_1 and Cy_2, *F* and *Fs* (cf. WINGE, 1936; LAMPRECHT, 1946) as well as Em_1 and Em_2 (DE HAAN, 1932). In such an investigation 9 : 7 segregating genes, e. g. *Wa* and *Wb* or *P* and *V*, should also be taken into consideration, since there is no fundamental differences between duplicate and complementary genes (cf. EYSTER, 1934, pp. 311—312). The possibility of a gradual divergence in the course of evolution between originally identical genes distributed over different members of a repeat (cf. WHITE, 1945, p. 48) should also be accounted for, and an absolutely similar effect of the corresponding genes could not be expected.

It is very likely that Cy_1 is situated between *F* and *St*. If the assumption of about 40 per cent crossing-over between *Wa* and *Gp* should turn out to be true, Cy_2 would probably be situated in the neighbourhood of *Fs*. According to LAMPRECHT (1942 a), *Gp* belongs to a linkage group of the constitution *Gp*—3,2 %—*Cp*—32,2 %—*Fs*—< 1 %—*Ast*, the percentage of crossing-over between *Gp* and *Fs* being 37,1. A duplication with segments containing respectively *F* Cy_1 and *Fs* Cy_2 would then most probably exist.

LAMPRECHT (1946) from quite different suppositions postulates the existence of small homologous segments in these previously discussed regions of non-homologous chromosomes. In a lecture at the Mendelian

Society, in Lund, in the autumn of 1944 NILSSON (unpubl.) reported that he had detected linkage between a point of translocation and the genes *St-B* in lines originating from the variety Extra Rapid. LAMPRECHT (1946) has been able to locate the point of translocation to the segment between *F* and *St* of the *B* chromosome and *Fs* and *Ast* of the *Gp* chromosome. The translocation studied by him is most probably identical with that investigated by NILSSON. LAMPRECHT further suggests that the appearance of semisterile plants, such as frequently occur in *Pisum*, are due to crossing-over between small homologous segments of non-homologous chromosomes. He also claims that the well-known figure of eight in *Pisum* (SANSOME, 1932) supports this duplication hypothesis. It should be pointed out that SANSOME himself is aware that this explanation is an alternative to the one generally accepted. A circumstance of interest in this connection is that the so-called interstitial segment in the figure of eight must be of considerable length and even allow of two chiasmata being formed (SANSOME, 1932). Anyhow, it would be of interest to determine the genes concerned.

Provided *Cy*$_2$ is really situated in the way suggested a lot of interesting problems will arise. We have not far to look for an explanation of genetically changed linkage values between *Cy*$_2$ and *Wa* in a possible inversion of the *Cy*$_2$—*Fs* region. The relations between the *Lc* and *Ld* factors described by DE HAAN (1930) and the *Cy* factors ought to be thoroughly studied. If new members of the *Cy* polygenes should thereby be detected, this might be of importance with respect to the duplication hypothesis. Further, it should be of interest to investigate the reasons that *Cy*$_1$ and *F* are only represented by two alleles while three multiple allelomorphs are known of *Cy*$_2$ (LAMM, 1937), *Wa* (WELLENSIEK, 1928) and *Fs* (LAMPRECHT, 1942). *Td*, according to LAMPRECHT (1945), is also probably represented by three allelomorphs. As, however, the position of *Cy*$_2$ is not definitely determined, these speculations are perhaps idle, and are mainly presented in order to emphasize the possible importance of future investigations.

The behaviour of the *Cy* factors is also interesting from the point of view of variation and selection of polygenic characters. I have the impression that dwarfs dominant for either *Cy*$_1$ or *Cy*$_2$ are most common, which is in line with the theories of polygenic inheritance given by MATHER (1941). Exact studies of these conditions would, however, be rather difficult and tedious.

I wish to express my gratitude to the State Horticultural Research Station at Alnarp for granting me the loan of experimental grounds and

of manual help, and address these thanks to the Head of the Institute, Professor F. NILSSON. I am also indebted to several colleagues mentioned in this paper for supplying me with material. To many other persons I also wish to express my thanks for valuable assistance and help in various ways.

SUMMARY.

In the present investigation of the linkage relations of the *Cy* factors in *Pisum sativum* the following main results have been obtained.

(1) The length factor Cy_1 is closely linked with *St*.

(2) The length factor Cy_2 is closely linked with *Wa*. It gives independent segregation with *A*, Cy_1, *D*, *I*, *Le*, *N*, *Wb* and *Wlo* but may perhaps belong to the *Gp* group.

(3) If Cy_2 and *Gp* are coupled in the way assumed in this paper, this would indicate that *Pisum sativum* has a duplication. This must not, however, be taken for granted until actually proved.

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ABSTRACTS — KURZE MITTEILUNGEN

GUDMUND SMITH: Psychological tests with twins.

On a material of 16 uniovular and 10 binovular pairs of twins of ages between 9 and 44 years (SMITH, 1946) a series of tests was performed to determine how a given objective environment is undergone and moulded by different individuals. Could it be expected that the subjective — and at the same time the significant objective — environment would be the same for all when the objective environment was the same? If this was not the case, would the subjective environments then show more agreements in individuals possessing similar hereditary factors than in those having different factors? As the answering of these questions required a rather intimate knowledge of the persons being tested, the characterological structure of these was determined by means of casuistic studies as well as modified Wartegg tests (WARTEGG, 1929).

In the first section of the test-series the objective environment consisted of a number of incomplete pictures, sketched or coloured, which the individuals under test were asked to interpret. In the second section it consisted of a puzzle with 13 combination possibilities which the subject had to set up in the way he thought most natural. The pictures were taken from O. MÜLLER (1929) and WEIL (1929), the idea of the puzzle from G. H. FISCHER (1944). In many respects the results show so great a variation that the first question can be answered straightaway: each individual selects or forms an individual environment out of the given objective environment.

On comparison of uniovular with binovular twins it was observed that the former agreed more often in their subjective environment than the latter. The interpretations and combinations made by uniovular twins were at times even almost identical. The agreement between them, however, was not found throughout the whole material. Four of the 16 pairs showed distinct discordance in the interpretation tests. In certain details of the picture material all the pairs of twins displayed concordance. But there, the inter-individual variation ceased, and hence no twin discordance is to be expected.

A close analysis of the results shows that the environmental discordance is not an accidental one but corresponds to a discordance in characterological structure. This applies not least of all to uniovular twins, whose individual choice of environment in the tests often appeared as divergences of interest in their natural environment. The question of the ultimate causes of this difference in character pattern — and consequently in environment and development — cannot yet be definitely answered. The working hypothesis must for the present be that a small difference in character — perhaps already induced by intra-uterine influences of environment, or by something else — produces an environmental discordance, which accentuates the difference in character and so on to a constantly increasing extent; as years pass only few pairs retain all their original similarity.

A comparison between various test-results within the pairs is also of interest. The discordance disclosed in the interpretation test by a uniovular pair need not be repeated in the combination test. Discordance may appear

or not, according to the aspect chosen for study and the objective environment into which the pair is introduced. Moreover, all uniovular twins do not show — as is evident from the above — the same differences, such being found to vary with each tested pair.

Some of the statements made on the strength of these test-results may seem rather self-evident, viz. that the discordance between uniovular twins varies from pair to pair and that different twins obtain dissimilar personal and, as we calculate, significant objective environments. There are, however, many scientists who are more or less unwilling to take such everyday observations into due consideration. Both BOUTERWEK (1935) and ECKLE (1939) — the latter more cautiously — generalize discordance in character under the antithesis »firm—loose». And those engaged in the mathematical calculation of the influence of hereditary and environmental factors upon variation often assume the same middle environmental difference for uniovular as well as binovular pairs. This material shows indeed that binovular pairs display a much greater one.

The investigation also rendered it possible to evaluate the specimens employed as to their original quality as test-material for JAENSCH's integration typology. They can scarcely be said to give unambiguous results.

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BÖRJE LÖVKVIST: Chromosome studies in *Cardamine*.

The following study began some years ago and was made in order to elucidate the relationships between *Cardamine pratensis* and *C. dentata*.

By means of cytological investigations on several hundred plants of this material collected in nature numerous chromosome races were found. *Cardamine pratensis* and *C. dentata* are probably the extremes in a series of types having more or fewer traits from the one or the other of the two species. As yet it is impossible to say anything about the taxonomy of this species complex.

Investigations of plants from the southern part of Sweden demonstrated a great variation in chromosome number. Somatic studies resulted in the distinction of the following races: $2n = 30$, 56, 60, 64, 68, 72 and 76. Plants

with 58 and 84 chromosomes were found, but not in so high a frequency that they deserve to be regarded as regular races. Plants with $2n = 30$, 56 and 76 have been studied in meiosis also. Only in those with the lowest number have meiotic irregularities as yet been found.

Plants of different races very often grow together in meadows, but always in a regional distribution, with the lowest number in only higher parts and with the races $2n = 56$, 60, 64 and 68 in lower parts, and the races with 72 and 76 near or in the water. Some examples can be given here.

Locality	Chromosome number in higher parts	lower parts	near water
Fågelsång	30	—, 60, —, 68	72, 76
Kungsmarken	30	56, —, —, 68	72, 76
Kalthus	30	—, 60, 64, —	—, —
Böljanemosse	—	56, 60, 64, 68	—, 76
Revinge	30	— ,—, —, —	72, —
Värpinge	30	56, 60, 64, —	—, —
Revlingemosse	—	—, —, 64, —	72, —

The conclusion drawn from this investigation is that in the present case higher chromosome number is correlated to higher water content of the soil.

Lund, Institute of Genetics, January, 1947.

ANTERO VAARAMA: Contributions to the cytology of the genus *Berberis*.

In the large genus *Berberis* the dominating chromosome number seems to be $2n = 28$ (TISCHLER, 1928, 1929; DERMEN, 1931; GIFFEN, 1936). The primary basic number has been supposed to be $x = 7$. Consequently the species with 28 chromosomes are tetraploids. Only in two species, *Berberis buxifolia* (TISCHLER, 1928; GIFFEN, 1936) and *B. turcomanica* var. *integrifolia* (DERMEN, 1931), has the chromosome number $2n = 56$ been counted. In regard to the basic number 7, these are octoploids.

During my stay in the Cyto-genetic Laboratory of Svalöf in the summer of 1945 I had an opportunity to examine some species and hybrids of the group of evergreen barberries in prepared material kindly placed at my disposal by the Head of the Laboratory, Dr. ALBERT LEVAN. The fixation of the root-tips was performed in the Ramlösa Plant Nurseries (Hälsingborg, Sweden), whose Director, Mr. HOLGER JENSEN, had kindly afforded facilities for this work. The *Berberis candidula* material, however, is from the plant nursery of the Alnarp Horticultural Institute (Åkarp, Sweden).

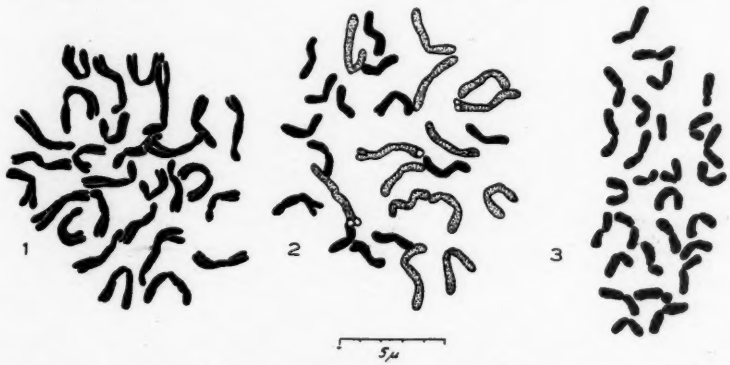
The only fixative used was the craf-modification commonly used in Svalöf. The paraffin sections were stained partly with FEULGEN, partly using the common iodine-gentian-violet method. It was found that the *Berberis* chromosomes are rather hard to stain. To obtain satisfactory results it was necessary to stain for 30 minutes in gentian-violet.

In the root-tip mitoses of the following seven *Berberis* species the chromosome number $2n = 28$ was counted: *Berberis candidula* SCHNEID., *B. Gagne-*

painii SCHNEID., *B. ilicifolia* FORST., *B. Julianae* SCHNEID., *B. Sargentiana* SCHNEID., *B. stenophylla* LINDL., *B. verruculosa* HEMSL. and WILS. All numbers are equal to those counted earlier from the same species (DERMEN, 1931; GIFFEN, 1936).

The number $2n = 56$ was observed in the following plants: *Berberis buxifolia* var. *nana* SCHNEID. and *B. actinacantha* MART. As mentioned above, the pure species *B. buxifolia* POIR. (TISCHLER, 1928; GIFFEN, 1936) has the same number. The chromosome number of the variety *nana* and the species *B. actinacantha* was not known earlier.

Among the material were also root-tips from the genus hybrid *Mahonia aquifolium* × *Berberis candidula* (= *Mahoberberis aquicandidula*), an artificial cross made by Mr. HOLGER JENSEN at Ramlösa. Two other *Mahonia—Berberis* crosses were previously known, viz. the old *M. aquifolium* × *B. vulgaris* (= *Berberis Neubertii*) and *M. aquifolium* × *B. Sargentiana* (LEVAN, 1944), the earlier successful cross of Mr. JENSEN.



Figs. 1—3. — Fig. 1. *Berberis candidula*: $2n = 28$. — Fig. 2. *Mahonia aquifolium* × *Berberis candidula*: $2n = 28$. The 14 short *Mahonia* chromosomes are black, the 14 long *Berberis* chromosomes are dotted. — Fig. 3. *Mahonia aquifolium*: $2n = 28$ (from LEVAN, 1944). — × 3000.

It was very interesting to see whether the individual size of the chromosomes as determined by LEVAN (l. c.) in *M. aquifolium* × *B. Sargentiana* was also maintained in the present case.

The chromosome number of *Mahoberberis aquicandidula* is $2n = 28$ and is thus the same as that of the parent species. A closer examination reveals that the chromosomes of *B. candidula* are partly median, partly submedian. The length of the chromosomes varies from 2,5 to 4,2 μ. Of the chromosome complement two pairs have a length of 4,0—4,2 μ, four pairs 3,3—3,5 μ, three pairs 3,0—3,2 μ, four pairs 2,7—2,8 μ and one pair 2,5 μ (Fig. 1).

Even this superficial survey of the chromosome morphology shows that if the haploid chromosome set of *B. candidula*, $n = 14$, is composed of two different basic sets of seven, in any case the sets differ from each other. Autotetraploidy is, therefore, out of question.

The chromosome size of *Mahonia aquifolium* varies between 1,5—2,5 μ (LEVAN, 1944). The length of five pairs is 2,3—2,5 μ, the others being smaller (Fig. 3).

The chromosomes of the hybrid can be divided on the basis of their length into two distinct groups (Fig. 2, black and dotted chromosomes). Among the group of the 14 longer chromosomes the following lengths can be found: 2 units about 4 μ, 4 units of 3,5—3,8 μ, 3 units of 3,2—3,3 μ, 4 units of 2,7—3,0 μ and one unit of 2,6 μ. Among the group of 14 shorter chromosomes there are 5 units of 2,4—2,6 μ, the others being 2,3—1,8 μ.

As can be seen, the length-classes of the hybrid correspond very well to those found in the parents. The chromosomes of the hybrid seem, however, to be somewhat longer than those of the parents. This may be due to the differences in fixation, but another possible cause is the different spiralization in the hybrid chromosomes. Anyhow, in the hybrid we are able to distinguish, except for one chromosome, the whole *Berberis* set from the chromosomes of *Mahonia*. The shortest *Berberis* chromosome belongs to the same length-class as five *Mahonia* chromosomes and is therefore impossible to identify.

At the same time as the conclusions of LEVAN (1944) are supported by the present observations, these are in accordance with the view that the individual size of the parent chromosomes is maintained in hybridization. Owing to the non-flowering of the *Mahonia—Berberis* hybrids it was not possible to study the properties of the meiosis. In the literature there are to be found many references to the unequal bivalents in analogous hybrids. The question was last treated by MEURMAN and SUOMALAINEN (1946). In their material, the hybrid *Montbretia*, besides unequal bivalents, they also observed unequal partners in the second division of meiosis.

In this connection I wish to present my hearty thanks to Dr. ALBERT LEVAN and Miss MAGNA PALM for all their kind help. I am also greatly indebted to Mr. HOLGER JENSEN and Dr. EMIL JOHANSSON for the material kindly provided by them.

State Horticultural Institute, Piikkiö, Finland

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